University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

THE ASSOCIATION BETWEEN HYPERTENSIVE DISORDERS OF PREGNANCY AND NEURODEVELOPMENTAL DISORDERS IN THE OFFSPRING

A thesis submitted to the National University of Ireland, Cork for the degree of

Doctor of Philosophy in the School of Public Health and

The Irish Centre for Maternal and Child Health Research

March 2020

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2020

Maher GM, O'Keeffe GW, Dalman C, Kearney PM, McCarthy FP, Kenny LC, Khashan AS. Association between Preeclampsia and Autism Spectrum Disorder: A Population-Based Study. **Journal of Child Psychology and Psychiatry.** 2020;61(2):131-9.

Maher GM, Dalman C, O'Keeffe GW, Kearney PM, McCarthy FP, Kenny LC, Khashan AS. Association between Preeclampsia and Attention Deficit Hyperactivity Disorder: A Population-Based and Sibling-Matched Cohort Study. **Acta Psychiatrica Scandinavica**. (2020) doi:10.1111/acps.13162.

Maher GM, Dalman C, O'Keeffe GW, Kearney PM, McCarthy FP, Kenny LC, Khashan AS. Association between Preeclampsia and Autism Spectrum Disorder and Attention Deficit Hyperactivity Disorder: An Intergenerational Analysis. **Acta Psychiatrica Scandinavica**. (2020) doi: 10.1111/acps.13180.

Maher GM, O'Keeffe GW, O'Keeffe LM, Matvienko-Sikar K, Dalman C, Kearney PM, McCarthy FP, Khashan AS. The Association between Preeclampsia and Childhood Development and Behavioural Outcomes. **Maternal and Child Health.** 2020;24(6):727-738.

2019

Maher GM, McCarthy FP, McCarthy CM, Kenny LC, Kearney PM, Khashan AS, O'Keeffe GW. A Perspective on Pre-eclampsia and Neurodevelopmental Outcomes in the Offspring: Does maternal inflammation play a role? **International Journal of Developmental Neuroscience**. 2019;77:69-76.

2018

Maher GM, O'Keeffe GW, Kearney PM, Kenny LC, Dinan TG, Mattsson M, Khashan AS. Association of Hypertensive Disorders of Pregnancy With Risk of Neurodevelopmental Disorders in Offspring: A Systematic Review and Meta-analysis. **JAMA Psychiatry.** 2018;75(8):809-19.

2017

Maher GM, O'Keeffe GW, Kenny LC, Kearney PM, Dinan TG, Khashan AS. Hypertensive Disorders of Pregnancy and Risk of Neurodevelopmental Disorders in the Offspring: A Systematic Review and Meta-analysis Protocol. **BMJ Open.** 2017;7(10):e018313.

LIST OF ORAL PRESENTATIONS

2019

Maher GM, O'Keeffe GW, Dalman C, Kearney PM, McCarthy FP, Kenny LC, Khashan AS. (2019) Association between Hypertensive Disorders of Pregnancy and Attention Deficit Hyperactivity Disorder: A Population-Based and Sibling-Matched Cohort Study [Oral Presentation], *Society for Social Medicine and Population Health & European Congress of Epidemiology Joint Conference 2019,* Cork, Ireland, 4-SEP-19 – 6-SEP-19.

Maher GM, O'Keeffe GW, Dalman C, Kearney PM, McCarthy FP, Kenny LC, Khashan AS. (2019) Association between Hypertensive Disorders of Pregnancy and Autism Spectrum Disorder: A Population-Based Study [Oral Presentation], *Society for Reproductive Investigation (SRI) 2019,* Paris, France, 12-MAR-19 – 16-MAR-19.
Awarded President's Presenter Award: This award recognises the most meritorious oral abstracts submitted to SRI's Annual Scientific Meeting by individuals still in training.

Maher GM, O'Keeffe GW, Dalman C, Kearney PM, McCarthy FP, Kenny LC, Khashan AS. (2019) Association between Hypertensive Disorders of Pregnancy and Autism Spectrum Disorder: A Population-Based Study [Oral Presentation], *SPHeRE Network 5th Annual Conference,* Dublin, Ireland, 26-FEB-19.

2018

Maher GM, O'Keeffe GW, Kearney PM, Kenny LC, Dinan TG, Mattsson M, Khashan AS. (2018) Hypertensive Disorders of Pregnancy and the Risk of Neurodevelopmental Disorders in the Offspring: A Systematic Review and Meta-analysis [Oral Presentation], *SPHeRE Network 4th Annual Conference,* Dublin, Ireland, 11-JAN-18.

2017

Maher GM, O'Keeffe GW, Kearney PM, Kenny LC, Dinan TG, Mattsson M, Khashan AS. (2017) Hypertensive Disorders of Pregnancy and the Risk of Neurodevelopmental Disorders in the Offspring: A Systematic Review and Meta-analysis [Oral Presentation], *INFANT Research Day 2017*, Cork, Ireland, 26-OCT-17.

Maher GM, O'Keeffe GW, Kearney PM, Kenny LC, Dinan TG, Mattsson M, Khashan AS. (2017) Hypertensive Disorders of Pregnancy and the Risk of Neurodevelopmental Disorders in the Offspring: A Systematic Review and Meta-analysis [Oral Presentation], *International Society for the Study of Hypertension in Pregnancy European Congress 2017,* Berlin, Germany, 6-SEP-17 - 9-SEP-17.

LIST OF KEY ABBREVIATIONS

ADHD Attention deficit hyperactivity disorder	**IUGR** Intrauterine growth restriction
ADDM Autism Developmental Disabilities Monitoring Network	**K-SADS-PL**=Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version
ADI-R Autism Diagnostic Interview, Revised	**MBR** Medical Birth Register
ADOS Autism Diagnostic Observation Schedule	**MCAR** Missing completely at random
aOR Adjusted odds ratio	**MCHRDB** Maternal and Child Health Research Database
APHP Alberta Perinatal Health Program	**MeSH** Medical subject headings
ASD Autism spectrum disorder	**mmHg** Millimetres of Mercury
ASQ Ages and Stages Questionnaire	**MNS** Midwives' Notification System
BMI Body mass index	**MODDS** Monitoring of Drugs of Dependence System.
CHARGE Childhood Autism Risks from Genetics and the Environment	**MOOSE** Meta-analysis of observational studies in epidemiology
CI Confidence interval	**NICU** neonatal intensive care unit
cOR Crude odds ratio	**NR** Not reported
CRP C-reactive protein	**OR** Odds ratio
DAG Directed acyclic graph	**PCR** Psychiatric Central Register
DDSN Department of Disabilities and Special Needs	**PDD-NOS** Pervasive Developmental Disorder - Not Otherwise Specified
DBP Diastolic blood pressure	**PIH** Pregnancy-induced hypertension
DSM Diagnostic and Statistical Manual of Mental Disorders	**PIN** Personal identity number
GIVM Generic inverse variance method	**PPV** Positive predictive value
GUI Growing Up in Ireland study	**PROSPERO** The International Prospective Register of Systematic Reviews
HDP Hypertensive disorder of pregnancy	**ROS** Reactive oxygen species
HR Hazard ratio	**RR** Relative risk
ICD International Classification of Disease	**RUPP** Reduced uterine perfusion pressure
IDA Integrated Database for Longitudinal Labour Market Research	**SDQ** Strengths and Difficulties Questionnaire
IL interleukin	**SGA** Small for gestational age
IP Index person	**SBP** Systolic blood pressure
IQ Intelligence quotient	**TNF** Tumor necrosis factor
IQR Interquartile range	**UK** United Kingdom
ISSHP International Society for the Study of Hypertension in Pregnancy	**US** United States

DECLARATION

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

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Signed Date

ACKNOWLEDGEMENTS

I would like to thank my amazing supervisory panel, Dr. Ali Khashan, Dr. Gerard O'Keeffe, Prof. Patricia Kearney and Prof. Louise Kenny. Thank you also to Dr. Fergus McCarthy and Prof. Christina Dalman for their fantastic guidance and support. I have always felt incredibly lucky to have worked with such a strong, and kind team. I will be forever grateful to you for being so generous with your expertise and time, and for providing me with invaluable feedback throughout my PhD project.

I would also like to thank Henrik Dal for providing me with data management support and advice on many occasions, and to everyone at the Epidemiology of Psychiatric Conditions, Substance use and Social Environment (EPiCSS) research group at Karolinska Institutet, Stockholm for being so friendly, and making me feel so welcome when I visited for my international placement.

Thank you to everyone within the School of Public Health and INFANT Research Centre for always being so kind, and willing to chat. Thank you also to Dr. Linda O'Keeffe for introducing me to linear spline multilevel modelling.

Of course, a special thank you to the Health Research Board and SPHeRE Programme for awarding me a place on the PhD programme, and to Claire Collins who was always a great help with all things SPHeRE.

Finally, thank you to my family for all your support. Thank you to Sharon for allowing me to talk things through, to Pa for always being so proud, and to Michał for agreeing to play PlayStation so I could study in peace! I would also like to thank my parents, especially my mother, Siobhán, for always being ready to drop everything in order to read my work, and for always placing such a high value on education.

Thank you all!

“I’m still learning”

Michelangelo, at age 87

THESIS ABSTRACT

Background and aims: Hypertensive disorders of pregnancy (HDP) are one of the most common gestational complications. HDP may be chronic (pre-dating pregnancy or diagnosed before 20 weeks' gestation) or arise *de novo* (either preeclampsia or gestational hypertension). Of these, preeclampsia is one of the leading cause of maternal mortality and morbidity. Evidence suggests an association between HDP and neurodevelopmental outcomes; however, results are limited and inconsistent. The aim of the current thesis was to examine the association between HDP (in particular, preeclampsia) and neurodevelopmental disorders in the offspring, including autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), child development and behavioural outcomes. This would be achieved by systematically reviewing existing literature, and conducting a range of robust analyses using Swedish National Registry data, and data from a nationally representative study of children living in Ireland.

Structure and methods: This thesis includes a brief introductory chapter (Chapter 1), and a detailed methods chapter describing study designs, data sources, exposure and outcome variables, statistical modelling, and the role of bias, confounding and chance in epidemiology (Chapter 2). Published literature on the relationship between HDP and neurodevelopmental disorders in the offspring were synthesised using a systematic review and meta-analysis, based on a pre-prepared protocol (Chapters 3 and 4). This was followed by a narrative literature review to provide a perspective on how maternal inflammation may lead to altered neurodevelopmental outcomes in preeclampsia-exposed offspring (Chapter 5).

Data from Swedish National Registers were analysed to examine the association between preeclampsia and ASD and ADHD, using Cox proportional hazards

regression analysis, adjusting for several perinatal and sociodemographic factors. Sibling-matched analysis was used to also control for shared genetic and familial confounding (Chapters 6 and 7). These associations were further explored by examining the intergenerational association between preeclampsia and ASD and ADHD (Chapter 8). Data from Growing Up in Ireland (GUI), a nationally representative study of children living in Ireland, were analysed to examine the association between preeclampsia and child development using the Ages and Stages Questionnaire (ASQ) at age 9-months, and behavioural outcomes using the Strengths and Difficulties Questionnaire (SDQ) at age 3 years, 5 years and 7-8 years. Multivariate logistic regression, linear regression and linear spline multilevel modelling were applied for this analysis (Chapter 9). Finally, the systematic review and meta-analysis was updated and included in this thesis (Chapter 10), along with discussion of findings, strengths and limitations of the thesis, and recommendations for future research (Chapter 11).

Results: *Updated systematic review and meta-analysis:* Among ASD studies, adjusted pooled results indicated that exposure to HDP is associated with a 33% increased odds of ASD when compared to those unexposed (OR: 1.33, 95% CI: 1.17, 1.52). Results of a subgroup analysis, examining a preeclampsia-ASD relationship in isolation provided an OR of 1.36 (95% CI: 1.18, 1.58), while the other HDP-ASD relationship was statistically non-significant with an OR of 1.29 (95% CI: 0.97, 1.71). Among ADHD studies, adjusted pooled results suggested that offspring exposed to HDP are 26% more likely to have ADHD compared to those unexposed (OR: 1.26, 95% CI: 1.15, 1.38). For the subgroup analysis examining the preeclampsia-ADHD relationship, the OR was 1.23 (95% CI: 1.13, 1.35), and for other HDP-ADHD relationship, the OR was 1.80 (95% CI: 1.25, 2.59).

Swedish National Registers: The adjusted Cox model suggested that preeclampsia was associated with 25% increased odds of ASD (Hazard ratio (HR): 1.25, (95% CI: 1.19, 1.30). The sibling-matched analysis reduced the HR to 1.17 (95% CI: 1.06, 1.28). The HR for preeclampsia and SGA combined (used as a crude proxy for preeclampsia with placental dysfunction) was 1.66 (95% CI: 1.49, 1.85) in the adjusted Cox model and 1.95 (95% CI: 1.53, 2.48) in the sibling-matched analysis. In the adjusted Cox model, preeclampsia was associated with a 15% increase in likelihood of ADHD (HR: 1.15, 95% CI: 1.12, 1.19), while the HR for preeclampsia and SGA combined was 1.43 (95% CI: 1.31, 1.55) in the adjusted model, compared to those unexposed to preeclampsia/SGA. The sibling-matched analysis did not materially change these associations.

Similar to the findings outlined above, the intergenerational analysis suggested that exposure to preeclampsia was associated with an increased likelihood of ASD and ADHD in offspring. In addition to this, results suggested that preeclampsia in both the child's mother and grandmother was associated with a 58% increased likelihood of ASD (HR: 1.58, 95% CI: 1.02, 2.46) and 34% increased likelihood of ADHD (HR: 1.34, 95% CI: 1.01, 1.80) in the child.

GUI study: Multivariate logistic regression suggested that preeclampsia was not associated with failing any ASQ domain. Preeclampsia was associated with abnormal SDQ cut-off of Emotional (score of ≥5) and Hyperactivity (score of ≥7) domains at age 5 years only. In the linear spline model, mean SDQ score was higher at age 3, 5 and 7-8 years in exposed groups, however did not always reach statistical significance.

Conclusions: This thesis rigorously investigates the association between HDP (in particular, preeclampsia) and neurodevelopmental disorders in offspring using a range of analytic approaches, and adjusting for a wide variety of potential confounders.

Pooled estimates from previous literature suggested an association between HDP and ASD and ADHD. Furthermore, the data from Swedish National Registers indicate that exposure to preeclampsia or preeclampsia and SGA combined (i.e. SGA baby exposed to preeclampsia) was associated with ASD and ADHD. The stronger association with preeclampsia and SGA combined than preeclampsia alone suggests that placental pathology may be a mechanism for the increased likelihood of ASD and ADHD. Results of the current thesis also suggest that preeclampsia may be associated with adverse neurodevelopmental outcomes across generations.

While we did not find strong evidence of associations between preeclampsia and child developmental and behavioural outcomes overall in the GUI study, exposure to preeclampsia was associated with an increased likelihood of subtle behavioural issues in the emotional and hyperactivity domain of the SDQ.

The overall conclusion of this thesis suggests an association between HDP and neurodevelopmental outcomes in offspring. It is important to note however, that we cannot rule out the presence of residual confounding in observational studies.

Chapter 1: INTRODUCTION

1.1 Introduction

1.1.1 Hypertensive Disorders of Pregnancy

Hypertensive disorders of pregnancy (HDP) are among the most common complications of pregnancy, estimated to effect up to 10% of all pregnancies[1]. They are classified by the International Society for the Study of Hypertension in Pregnancy (ISSHP) as "chronic hypertension", "white-coat hypertension", "masked hypertension", "transient gestational hypertension", "gestational hypertension" and "preeclampsia" (*de novo* or superimposed on chronic hypertension)[2] (**Figure 1.1**) (figures and tables are located at the end of each chapter).

Chronic hypertension refers to a diagnosis of hypertension (defined as systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 mmHg) before pregnancy or within the first 20 weeks of pregnancy. White-coat hypertension refers to elevated blood pressure during a clinic visit (≥140/90mmHg) but normal blood pressure measured at home or work (<135/85mmHg); while masked hypertension is a form of chronic hypertension that refers to blood pressure readings that are normal at a clinic visit, however elevated at other times[2].

Transient gestational hypertension, gestational hypertension and preeclampsia are new onset hypertension (blood pressure ≥140mmHg systolic or ≥90mmHg diastolic) typically arising at or after 20 weeks of pregnancy[2]. Transient gestational hypertension generally arises in the second or third trimester but resolves without any treatment. Gestational hypertension is persistent hypertension that develops without features of preeclampsia, while preeclampsia can be *de novo* or superimposed on chronic hypertension (i.e. women with chronic hypertension who develop preeclampsia). Of these, preeclampsia is one of the leading causes of maternal morbidity and mortality[2, 3].

1.1.2 Preeclampsia

Preeclampsia, which affects approximately 5% of all pregnancies[4] is a serious obstetric complication, and is responsible for more than 500,000 fetal and neonatal deaths, and 70,000 maternal deaths each year, worldwide[2].

Past hypotheses of preeclampsia have varied considerably, and are thought to date back to 400BC when it was hypothesised that preeclampsia was a result of an imbalance in the four humors (blood, phlegm, yellow bile, and black bile)[5].

The introduction of the word 'eclampsia' (derived from the Greek word eklampsis, meaning lightning) did not appear until 1619 however, and was thought to be a form of epilepsy. It was not until the 18th century when eclampsia was differentiated from epilepsy by Boissier de Sauvages, an important difference being the nature of convulsions (acute in the case of eclampsia, and chronic in epilepsy with convulsions recurring over time)[5, 6].

The late 1800s saw the theory of toxins emerge where it was believed that increased secretion of waste and debris, for example from the bowels, and maternal and fetal systems, would prevent accumulation of toxins in the blood that could potentially lead to convulsions. As a result, treatment for pregnant women with headaches and edema involved bleeding and purging[5].

Classification of the disorder was refined throughout the late 18th and 19th century, as symptoms such as headache, temporary loss of vision, severe stomach pain, and edema in the upper body became recognised as a preeclamptic state (before convulsions) that should also heed the attention of clinicians. However, it was not until the introduction of Scipione Riva-Rocci's mercury sphygmomanometer in 1896, allowing the measurement of systolic blood pressure, that increased understanding that preeclampsia was a hypertensive disorder[5, 7].

To this day, the exact etiology of preeclampsia remains unknown. However, it is understood that the placenta can play a role in pregnancies complicated by preeclampsia through the release of vasoactive factors into the maternal circulation. Such factors include soluble fms-like tyrosine kinase-1 (sFlt-1), cytokines, angiotensin II and type 1 receptor autoantibodies which target the maternal vascular endothelium, resulting in the clinical manifestations of the disorder[6]. These clinical manifestations, as recently redefined by the International Society for the Study of Hypertension in Pregnancy (ISSHP), include hypertension (blood pressure ≥140/90mmHg at or after 20 weeks' gestation) accompanied by one or more of the following:

1. Proteinuria.

2. Maternal organ dysfunction, including:

- Acute kidney injury (creatinine > 90umol/L; 1mg/dL).
- Liver involvement (elevated transaminases, for example alanine aminotransferase (ALT) or aspartate transaminase (AST) >40 international units/litre (IU/L)) with or without right upper quadrant or epigastric abdominal pain).
- Neurological complications (for example, eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, persistent visual scotomata)
- Haematological complications (thrombocytopenia – platelet count below 150,000/uL, disseminated intravascular coagulation (DIC), hemolysis).

3. Uteroplacental dysfunction (such as fetal growth restriction, abnormal umbilical artery Doppler wave form analysis, or stillbirth)[2].

Despite the precise etiological mechanisms of preeclampsia yet to be uncovered, several risk factors such as prior preeclampsia, chronic hypertension, multiple

gestation, pre-gestational diabetes, maternal obesity, and antiphospholipid antibody syndrome are well documented[2, 8].

Previous epidemiological studies have used varying data collection methods to determine if a diagnosis of preeclampsia was present, such as self-reported data[9-11], medical records[12, 13] and population-based registries[14, 15]. A common method of classifying preeclampsia in medical records and registry data is the International Classification of Diseases (ICD) coding system. This thesis uses data from Swedish-based registries, classifying preeclampsia according to the Swedish version of ICD-8 [code 637], ICD-9 [code 642], and ICD-10 [code O14 or O15][16] (chapters 6, 7 and 8). In addition, self-reported data on preeclampsia from a longitudinal study of children living in Ireland is also used (chapter 9).

1.1.3 HDP and neurodevelopmental outcomes

1.1.3.1 The Fetal Origins Hypothesis

The Fetal Origins Hypothesis which is associated with the English physician and epidemiologist David Barker, proposes that the *in utero* experience is a critical period and can result in lifelong consequences for the offspring[17]. While the hypothesis originally outlined a link between fetal undernutrition and coronary heart disease, it is now well recognised that the environment during fetal development may have an effect on a range of health outcomes, including neurodevelopment[18]. For example, previous literature suggests that *in utero* exposure to environmental toxicants[18], birth by caesarean section[19], and advanced maternal age are associated with adverse neurodevelopment[20]. Similarly, evidence alludes to an association between HDP and neurodevelopmental outcomes in offspring[21, 22].

1.1.3.2 HDP and Autism Spectrum Disorder

Two neurodevelopmental outcomes that can typically present during childhood are autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). ASD is characterised by persistent impairments in interpersonal interaction and restricted or repetitive patterns of behaviour[23]. Recent decades have observed a significant rise in the prevalence of ASD. For example, at the end of the 20th century, global prevalence was estimated to be approximately 0.62%[24]. However, the current global prevalence is approximately 1%, increasing to 1.5% in developed countries[25, 26].

Several possible explanations have been proposed to account for this rise, such as increased awareness of ASD, with an estimated 60% of the increase occurring as a result of changes in diagnostic criteria[27]. In addition to this, while there is a general consensus that genes play a major role in the development of ASD, the environmental contribution is estimated to be about 17-50%, however the potential etiological factors are not well understood[28, 29].

Previous literature suggests an association between HDP and ASD; however, findings are inconsistent. For example, two case control studies conducted in Sweden and California allude to a positive relationship between preeclampsia and ASD[13, 14], while a further two cohort studies, both of which were conducted in the United States, also suggest a significant association between preeclampsia and ASD[9, 30]. In addition to these, there are studies suggestive of a positive relationship, but fail to reach statistical significance[12, 31]. Conversely, other studies indicate a protective association between preeclampsia and ASD, however do not control for potential confounding factors[32-34].

Similarly, the epidemiological evidence examining other categories of HDP and likelihood of ASD is conflicted, with some studies indicative of a positive association. For instance, Curran et al. and Polo-Kantola et al. studied the association between HDP (which may have included preeclampsia) and ASD using data from the United Kingdom and Finland respectively, with both study authors finding significant positive associations[21, 35]. However, in contrast to this, Lyall et al. and Langridge et al. found an inverse association between pregnancy induced hypertension and ASD[9, 36].

1.1.3.3 HDP and Attention Deficit Hyperactivity Disorder

Attention deficit hyperactivity disorder (ADHD) is characterised by inattention, hyperactivity and impulsivity, and has an estimated global prevalence of approximately 5%[37, 38].

Akin to ASD studies, conflicting findings are observed amongst previous epidemiological literature examining a preeclampsia-ADHD relationship. There are studies that conclude positive associations[39-43]; Silva et al. and Getahun et al. (two large case-control studies from Western Australia and Southern California respectively), used existing medical records data to conclude a positive association between preeclampsia and ADHD. Furthermore, Mann and McDermott conducted a cohort study from Medicaid billing records and found a 20% increased odds of ADHD in preeclampsia-exposed offspring[41].

There are also studies that suggest a positive association, although span the null value[44-46], while conversely, there is evidence of a negative relationship between preeclampsia and ADHD[47, 48], but these studies do not control for potential confounders[47, 48]. While fewer studies examine a relationship between other

categories of HDP and ADHD, the presence of a positive association between self-reported HDP (which may include preeclampsia) and ADHD is outlined in Bohm et al.[22].

1.1.3.4 A Need for Further Research

In addition to conflicting results of studies examining a HDP-ASD and HDP-ADHD association, residual or unmeasured confounding is of particular concern in the literature. For example, two separate case-control studies[11, 49] and one cohort study[50] conducted in the United States, India and Canada examined the association between pregnancy induced hypertension and ASD but failed to control for potential confounding factors. Furthermore, a case-control study conducted in Poland investigating a chronic hypertension-ASD relationship, although matched on year of birth, sex and general practitioners, did not control for potential confounders in the analysis phase of the study[12]. Similar issues are observed among ADHD studies as validity of findings are often limited by residual confounding[42, 47, 48].

Furthermore, previous epidemiological literature on this topic does not control for genetic factors shared by siblings, a limitation that should be addressed before reaching conclusions that are more definitive. One such method to address this is a sibling-matched design. In brief, this method uses stratified Cox regression, and is an extension of the paired binomial model, taking into account different lengths of follow-up time. It can estimate the probability of ASD/ADHD within the family, while also taking account of unmeasured confounding factors shared by siblings, including family environment, lifestyle factors such as diet, maternal characteristics, and genetic factors[51].

Lastly, evidence suggests that certain non-communicable disease-risk may be intergenerational. For example, cardiovascular disease in grandparents is associated with congenital heart disease in grandchildren[52], while type 2 diabetes in one generation may be linked to birthweight across two subsequent generations[53]. However, whether there is any intergenerational link between preeclampsia and ASD and ADHD is unknown. Therefore, given the long-term consequences of preeclampsia for both mother and child[54-56], examining the intergenerational association between preeclampsia and ASD and ADHD is warranted.

1.1.3.5 HDP and Other Neurodevelopmental Outcomes

There is some evidence of an association between HDP and impaired cognitive functioning, behavioural issues and intellectual disability[57-59]. For example, some patterns of association can be observed between preeclampsia and cognitive impairment when confined to specific populations such as growth restricted, preterm and low birthweight babies[57, 60, 61]. Similarly, there is some epidemiological evidence that is suggestive of a potential link between HDP and intellectual disability[36, 62, 63]. However, there is a lack of overall consistent findings as some studies have not found significant associations[13, 22, 64, 65], while others suggest a protective association[66, 67]. In addition to this, much of the research examining a preeclampsia-child development relationship has been conducted on specific populations such as preterm and very low birthweight infants with small sample sizes. As a result, generalisability of findings is often limited, highlighting the need for further research in this area[57, 58, 61, 64, 68, 69].

1.1.4 Biological Mechanisms

Several potential mechanisms have been proposed in an attempt to explain the association between HDP and neurodevelopmental outcome (**Figure 1.2**). For example, placental dysfunction, associated with HDP, may result in suboptimal nutrient and oxygen availability for the fetus, potentially affecting the developing brain, and increasing the likelihood of adverse neurodevelopmental outcome[13, 30, 70-72].

Furthermore, the inflammatory response associated with preeclampsia could act as a mediator between preeclampsia and neurodiversity given that maternal inflammation is a recognised risk factor for adverse neurodevelopmental outcome[73-75] (see chapter 5 for a narrative review of evidence on how maternal inflammation may lead to altered neurodevelopmental outcomes in preeclampsia-exposed offspring). For example, research by Spann et al. suggests a negative association between maternal interleukin (IL)-6 (a pro-inflammatory cytokine) and brain connectivity in toddlers[76], while Rasmussen et al. has demonstrated an inverse association between maternal IL-6 and offspring cognition at 12 months of age[77]. In addition to this, a Finnish population-based study, with data on over one million pregnancies, have demonstrated how the inflammatory biomarker, C-reactive protein (CRP) which is associated with preeclampsia, is associated with a 43% increased risk of ASD in offspring[78, 79]. Likewise for ADHD, despite high heritability estimates, gene environment interactions may also play a role[80], and while fewer hypotheses have been put forward addressing the biological mechanisms of ADHD specifically, similar mechanisms may be involved[22, 81, 82].

1.1.5 Overall Aims and Objectives

Given the lack of overall consistent findings, and threats to validity, for example due to unmeasured and residual confounding, the overall aim of this thesis was to examine the association between HDP, (in particular, preeclampsia), and neurodevelopmental disorders in offspring (as outlined in **Figure 1.3**). Specifically, the objectives of the thesis were as follows:

1. Based on a pre-prepared protocol, synthesise the available published literature on the relationship between HDP and neurodevelopmental disorders in the offspring in the form of a systematic review and meta-analysis.

2. Review evidence and provide a perspective on how maternal inflammation may lead to altered neurodevelopmental outcomes (in particular ASD) in preeclampsia-exposed offspring.

3. Examine the association between preeclampsia and ASD using data from Swedish National Registers, controlling for several important confounders, including confounding due to shared genetics and familial environment through sibling-matched analysis.

4. Examine the association between preeclampsia and ADHD using data from Swedish National Registers, controlling for several important confounders including confounding due to shared genetics and familial environment through sibling-matched analysis.

5. Examine the intergenerational association between preeclampsia and ASD and ADHD using data from Swedish National Registers.

6. Examine the association between preeclampsia and child development, and behavioural outcomes using data from Growing Up in Ireland, a nationally representative study of children living in Ireland.

7. Update the systematic review and meta-analysis for ASD and ADHD, including the current results and any newly published studies.
(See **Figure 1.4** for publication progress).

Figure 1-1 Hypertensive Disorders of Pregnancy (as outlined by ISSHP)[2]

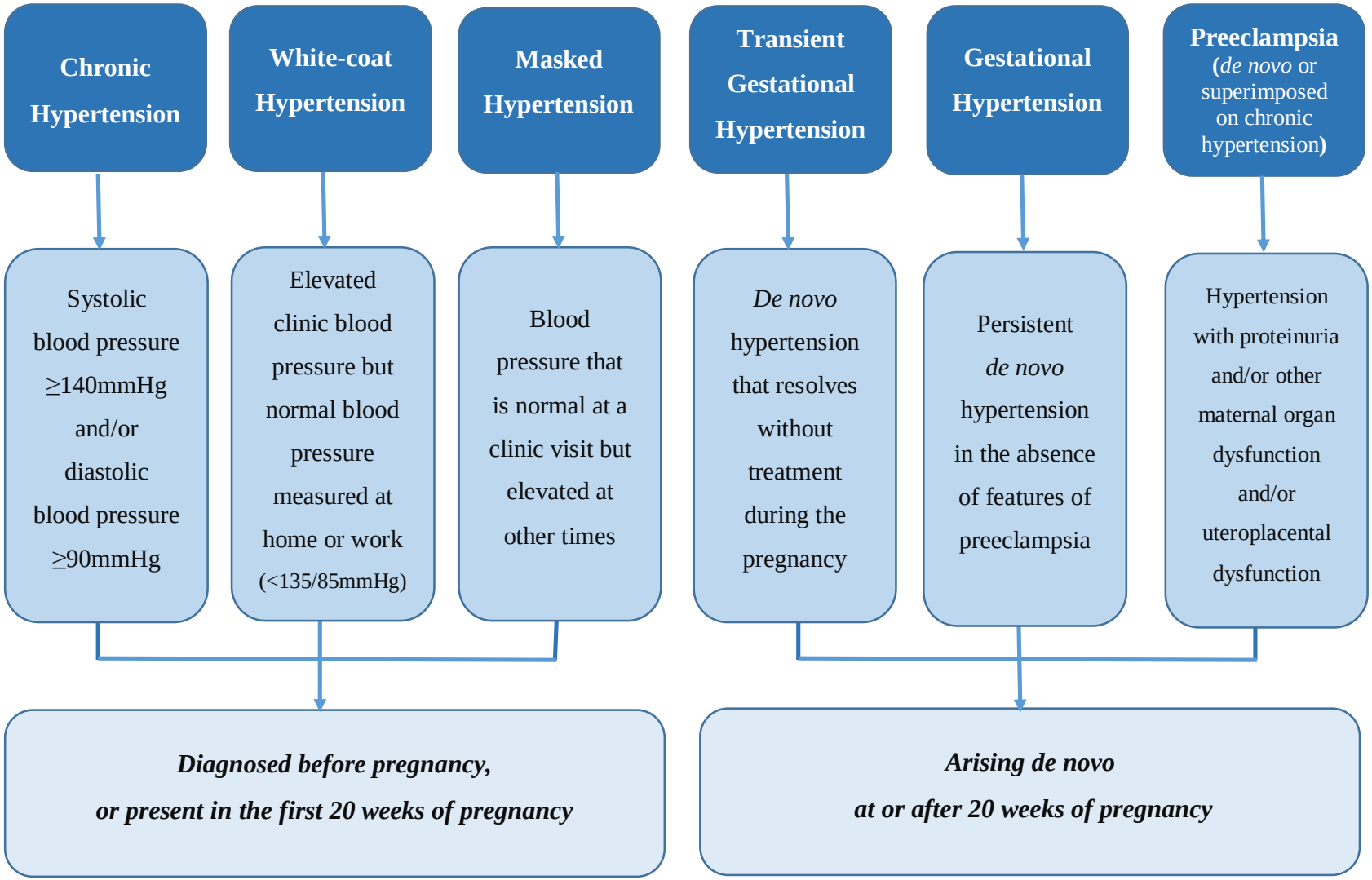


Figure 1-2 Proposed mechanisms for the association between HPD and adverse neurodevelopmental outcome

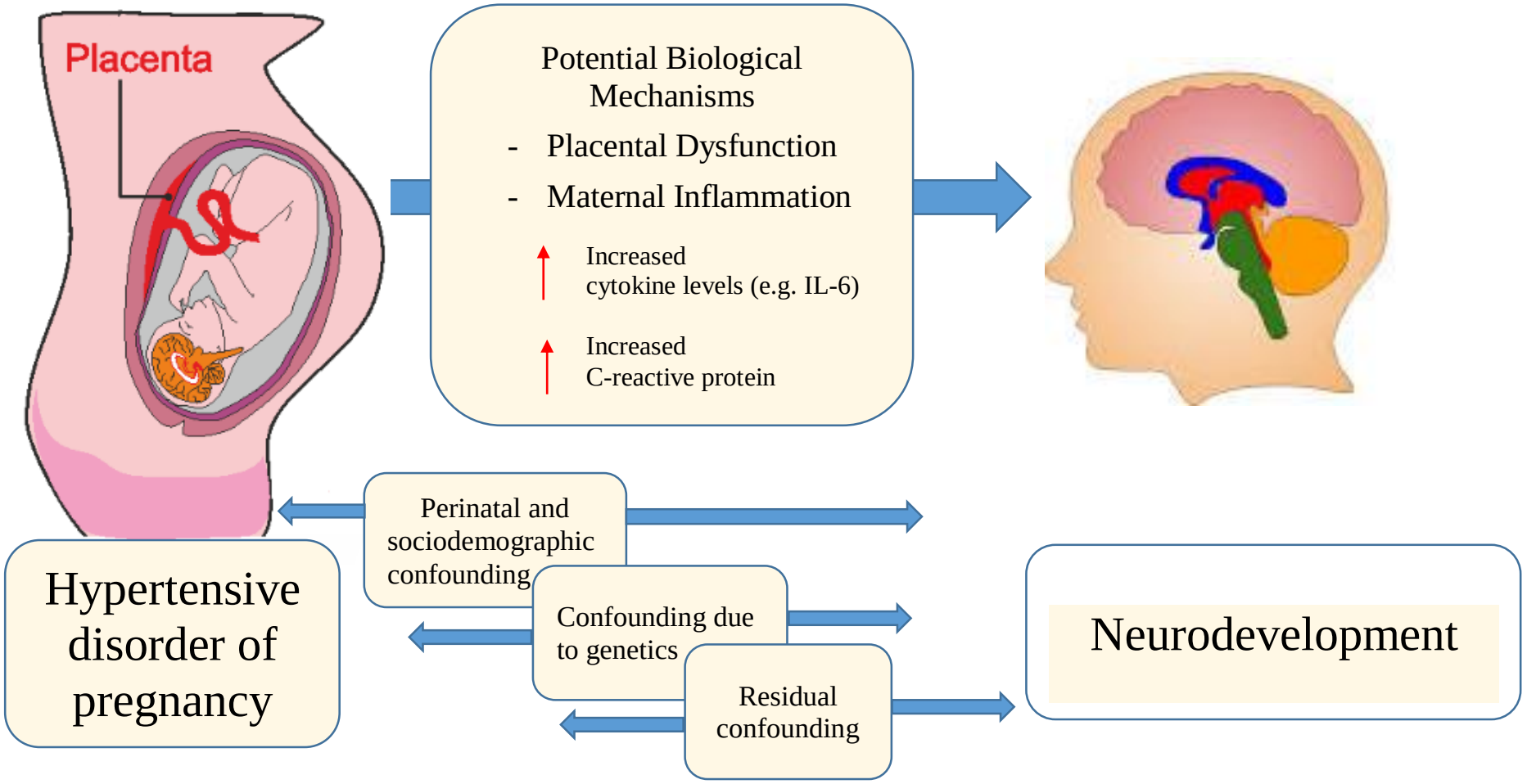


Figure 1-3 Thesis overview including overall aim, specific objectives and corresponding chapters

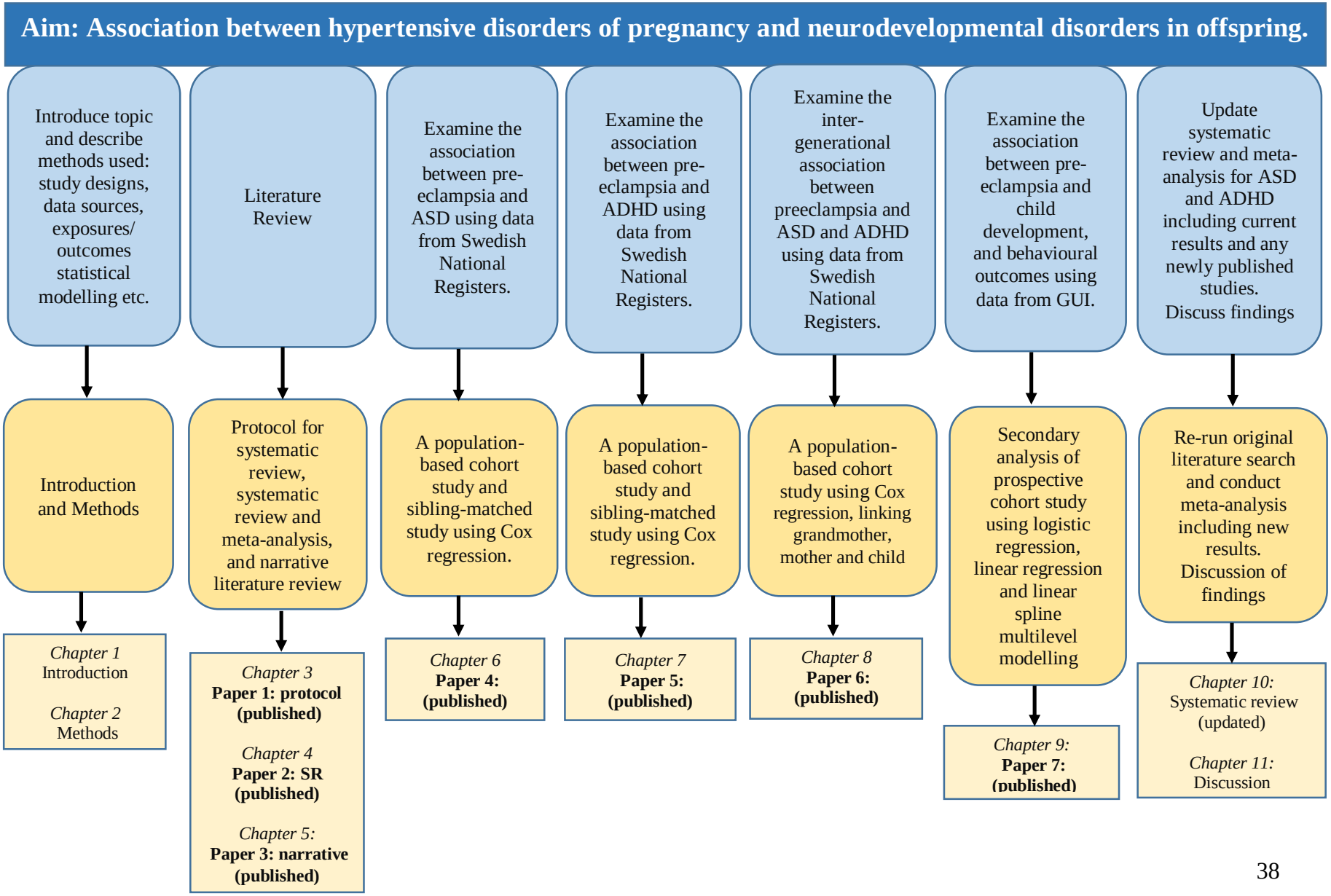
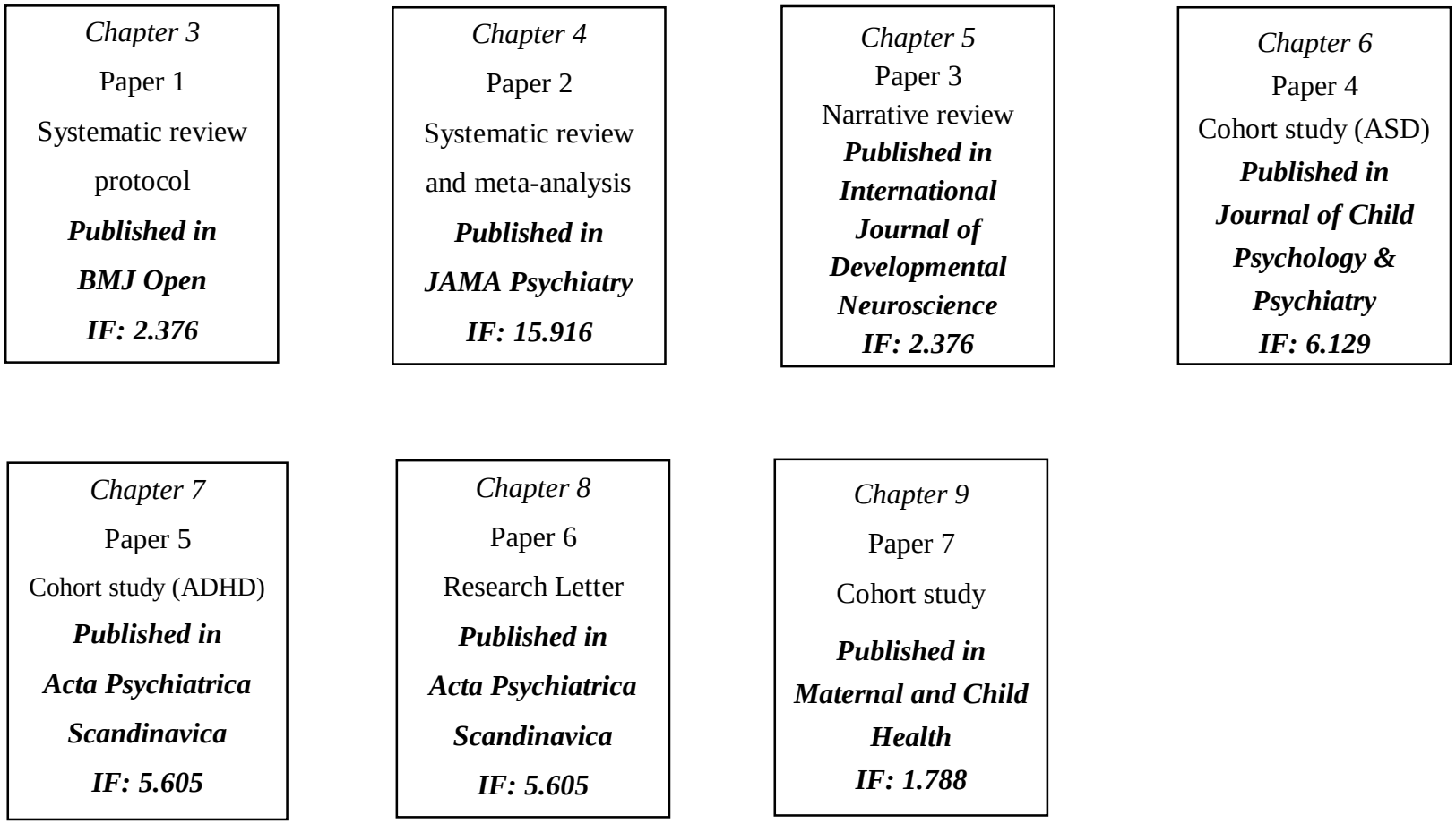


Figure 1-4 Publication progress to date



Chapter 2: METHODS

2.1 Chapter Overview

This chapter provides a brief outline of the methods used in this thesis (a full description of the methods used for each study can be found within each paper). In particular, this chapter describes the following:

2.2 Study designs used.

2.3 Data sources used (where applicable).

2.4 Main exposure and outcome variables used.

2.5 Statistical modelling used.

2.6 Bias, confounding and chance in epidemiology.

2.2 Study Design

2.2.1 Systematic Review Protocol

Chapter 3 is comprised of a systematic review protocol outlining the rationale, research question, and planned methods of the systematic review. This is to ensure the decisions guiding the systematic review were *a priori*, preventing arbitrary decisions, and ensuring a transparent process. In addition to publishing the review protocol in the BMJ Open[83], the protocol was registered on PROSPERO (registration number: CRD42017068258) to prevent unintended duplication by other systematic reviewers.

The following PICO was used to determine inclusion/exclusion criteria:

Population: Pregnant women and their children.

Intervention/Exposure: Diagnosis of any HDP (any definition in previous literature).

Comparison: No diagnosis of HDP.

Primary Outcomes: ASD and ADHD (any definition in previous literature).

Secondary Outcomes: Other neurodevelopmental disorders.

2.2.2 Systematic Review and Meta-analysis and Narrative Review

Chapter 4 is comprised of a systematic review and meta-analysis (based on the protocol in chapter 3). This was conducted to systematically locate and synthesise published epidemiological literature on the association between HDP and neurodevelopmental disorders in the offspring. The following PICO was used in the review:

Population: Pregnant women and their children.

Intervention/Exposure: Diagnosis of any HDP (any definition in previous literature).

Comparison: No diagnosis of HDP.

Primary Outcomes: ASD and ADHD (any definition in previous literature).

Secondary Outcomes: Neurodevelopmental and other behavioural or cognitive outcomes.

Data were analysed using Review Manager 5.3. Random-effects meta-analyses calculated overall pooled estimates of the relationship between combined HDP, preeclampsia only, and other HDP, and the outcomes of ASD and ADHD. We used the generic inverse variance method (GIVM) to allow studies that do not report raw data to be included in the meta-analyses. Forest plots were used to display crude and adjusted estimates for ASD and ADHD, while standalone estimates were reported for secondary outcomes.

Conversely, chapter 5 contains a narrative review summarising existing evidence of how maternal inflammation may lead to altered neurodevelopmental outcomes in preeclampsia-exposed offspring. This was to provide a perspective on a potential biological pathway between preeclampsia and neurodevelopmental outcome, in particular ASD.

2.2.3 Cohort Studies using Swedish National Registers

Chapters 6, 7 and 8 are comprised of population-based cohort studies, using Swedish National Registers. These chapters were informed by the systematic review in chapter 4 which identified several limitations of previous literature that should be addressed in future research studies. Therefore, the aim of these studies was to examine the association between preeclampsia and ASD, and preeclampsia and ADHD, while addressing the key limitations identified in the systematic review.

A prospective cohort study and case-control study are also viable options that could potentially address these research areas, and both have strengths and limitations. For example, prospective cohort studies recruit participants at baseline, (during which time no one has been diagnosed with the outcome of interest), and follows them forward in time to determine who experiences the outcome of interest. These type of studies can be used to calculate the incidence and prevalence of the study outcome, while it is also possible to collect information on multiple potential confounders, which can be easily adjusted-for in the analysis phase of the study. Furthermore, prospective cohort studies are flexible in that they can be used to examine the association between an exposure and multiple outcomes. Conversely, prospective cohort studies tend to be a lengthy process with long follow-up periods, therefore tend to be a more expensive option and generally not suited to studies with rare outcomes.

Case-control studies identify those with the outcome of interest, match them to suitable controls and examine past exposures. Case-control studies can be conducted faster than prospective cohort studies, and are therefore a less expensive option making them suitable for studying rare outcomes. However, case-control studies are particularly prone to selection bias due to issues in recruiting appropriate controls, while confounding in case-control studies may also be an issue. For example, if

confounding is addressed in the design phase of the study through matching, this should be taken into account in the analysis phase of the study by conducting conditional logistic regression. In addition to this, case-control studies cannot be used to estimate the incidence or prevalence of an outcome.

Retrospective cohort studies are similar to prospective cohort studies, however an important difference being that retrospective studies use historical data where the exposure and outcome of interest has already occurred in some individuals. A limitation of a retrospective study is that data on potential confounders is limited to what is available in the existing dataset. Moreover, if using existing data that was not collected to answer a specific research questions, the researcher cannot be certain of the processes used to collect the data.

A population-based cohort study, which contains some of the features of prospective and retrospective, was used for this thesis. For example, in a population-based cohort study, data are recorded prospectively, limiting the likelihood of recall bias; however it shares with the retrospective design the fact that you are limited to what variables are available in the dataset.

A population-based cohort study was chosen for the following reasons:

1. Swedish population-based registry data was available which could be used to address this piece of research, saving on resources such as time and financial cost associated with collecting primary data.

2. The registers contain data on a wide range of perinatal and sociodemographic factors, allowing adjustment for several potential confounders.

3. The large sample size (more than two million children) allowed the research to be conducted with sufficient power.

4. A personal identity number (PIN), assigned to each Swedish resident allowed for individuals and family members to be linked across national registers. Therefore, we were able to adjust for shared genetic and familial confounding using sibling-matched analysis, while also examine an intergenerational association between preeclampsia and ASD and ADHD.

2.2.4 Cohort Study using Growing Up in Ireland

Chapter 9 examines the associations between preeclampsia and child development and behavioural outcomes, and is a secondary analysis of the prospective cohort study, Growing Up in Ireland. Similar to above, a cohort study or case-control study are valid options to investigate this research topic. However, a secondary analysis of a prospective cohort study was used for the following reasons:

1. Data on exposures and outcomes of interest were readily available in the Growing Up in Ireland data, therefore collecting primary data, which can take several years and require large financial costs, was not necessary.

2. Data on behavioural outcomes were measured at several time-points, making a case-control study a less viable option.

3. National registers such as those in Sweden lack information on child development and behavioural outcomes; however, this information was available in the Growing Up in Ireland dataset, thus complementing the Swedish data studies.

4. Growing Up in Ireland also contains a wide range of perinatal and sociodemographic data, allowing adjustment for several potential confounders.

2.3 Data Sources

2.3.1 The Swedish National Registers

2.3.1.1 Personal Identification Numbers

Since 1947, every permanent resident of Sweden is assigned PIN when they register their birth, or move to Sweden and intend to stay for at least one year. This PIN is a unique number consisting of three parts: date of birth, a sex-specific three-digit birth number, and a 'check digit' that verifies the first two parts are correct. The PIN was originally introduced to identify individuals resident in Sweden. However, it is now also used as a means of linking individuals across national registers, allowing researchers to examine a vast range of research questions, including those that require long periods of follow-up[84].

2.3.1.2 The Medical Birth Register

Founded in 1973, the Medical Birth Register has data on over 96% of all births in Sweden, and contains information on prenatal care, delivery, neonatal care, as well as maternal sociodemographic and lifestyle factors. It is compulsory for all healthcare providers to report to the register, however, data on approximately 1-4% of births are either missing completely or incomplete[85].

While the basic structure of the register has not changed much since 1973, there have been some modifications to content and methods of data collection. For example, from 1973-1982 medical secretaries at obstetric clinics summarised medical records on standardised forms, which were then sent to the National Board of Health for computerisation. However, after a content evaluation in 1976, it was decided that basic records of antenatal care of the mother, delivery records, and infant examination records would be sent to the National Board of Health directly for inputting into the

Medical Birth Register, to prevent any discrepancies when transferring data. This new procedure came into effect in 1982, during which time the content of the register was also expanded to include information on maternal factors such as smoking status and body mass index (BMI)[86].

2.3.1.3 The National Patient Register

The National Patient Register was founded in 1964 when it started collecting information on somatic inpatient care in six Swedish counties. The register also contained information on inpatient psychiatric care from the 1960s. However, all psychiatric data originating before 1973 were removed when the register was reconstructed in the 1990s. During this time, the decision was made to include the PIN as the unique identifier in all hospital discharges and efforts were made to link earlier hospital discharges to the PIN. Therefore, the National Patient Register now contains information on inpatient psychiatric diagnoses from 1973, and has obtained complete national coverage from 1987[87].

Every year data on approximately 1.5 million discharges are collected. Each discharge record contains information on patient-related data (such as PIN, sex and age), data about the caregiver (such as hospital code and department), administrative data (such as admission and discharge date), and medical data (such as primary and additional diagnoses, which can be co-morbidities and/or complications of the primary diagnosis)[87]. Several validation studies of inpatient data in the National Patient Register have been conducted, and a review of these studies suggests high validity, concluding a positive predictive value of 85-95% for most diagnoses[87].

Since 2001, the National Patient Register was expanded to include outpatient data from both public and private caregivers, with increasingly better coverage until

2006[88]. In 2010, it was estimated that coverage of data from private caregivers was approximately 80%, coverage from public caregivers was almost 100%, while primary health care data are still not reported on a national level[87].

2.3.1.4 The Prescribed Drug Register

The Prescribed Drug Register was founded on 1st July 2005, and the unique PIN was used to allow linkage to other registers[89]. Prior to this, establishing a Prescribed Drug Register was met with political resistance as it was thought to be a threat to patient confidentiality more so than other national registers. However, lobbying from patients' and older people's organisations led to the introduction of the Prescribed Drug Register[90]. The register collects information on all dispensed prescribed drugs in outpatient care and coverage is almost 100% complete. It also contains information on dosage, age, sex, place of residence, date of prescribing and dispensing, healthcare provider and profession of prescriber for more than six million Swedish residents each year[89, 90]. However, the register does not contain information on over-the-counter medications, medications used in hospital care, and is not complete with regard to drugs used in nursing homes. In addition to this, the register does not collect information on indication for the prescription, or drugs that have been issued by physicians but not dispensed[90].

2.3.1.5 The Multi-generation Register

The first version of the Multi-generation Register was created in 2000, and replaced what was known as the Second Generation Register kept by Statistics Sweden from 1994-1999. It is part of the Total Population Register (see below) and is made up of people who have been registered in Sweden at some point since 1961, and those who

were born in 1932 or later. Each year, a new version of the register is created, and includes new people who immigrated, or were born during that year[91]. The purpose of the Multi-generation Register is to connect people with their biological parents, with information on mothers available in 97% of cases, and on fathers in 95% of cases[92].

2.3.1.6 The Total Population Register

The Total Population Register was created in 1968, and is maintained by the government agency Statistics Sweden. The register contains background information such as age, sex, municipality and country of birth, in addition to information on number of live births, stillbirths, deaths, immigrations and emigrations, migrations within Sweden, changes in civil status, and changes in citizenship. It is estimated that approximately 100% of births and deaths, 95% of immigrations and 91% of emigrations are reported to the Total Population Register within 30 days, with these figures increasing over time. However, underreporting of emigration data is estimated to be up to 0.5% of the registered total population in Sweden[93].

2.3.1.7 The Register of Education

The Register of Education contains data on graduation and educational background such as highest education level and completion year. As well as this, the register collects information on demographics including age, sex, and municipality of residence. The educational data contained in the register was updated using the Population and Housing Census 1990, and has improved the quality of the register from 1990 and onwards considerably[94].

2.3.2 Growing Up in Ireland

2.3.2.1 Study population

Growing Up in Ireland (GUI) is a nationally representative longitudinal study of children, funded by the Irish Government, carried out jointly by The Economic and Social Research Institute and Trinity College Dublin, and managed by the Department of Children and Youth Affairs and Central Statistics Office. The study, which started in 2006, follows two separate cohorts over a number of years: a child cohort, (which began 2006) when children were 9 years, and an infant cohort (which began in 2008) when children were 9 months[95].

The GUI study consists of various waves of data collection. This thesis uses data from waves 1-4 of the Infant Cohort only. Wave 1 (baseline) was conducted when the children were nine-months, wave 2 follow-up data were collected when the children were 3 years old, wave 3 follow-up data were collected when the children were 5 years old and wave 4 follow-up data were collected when the children were 7-8 years old. In waves 1-3, data were collected through face-to-face interviews conducted by trained interviewers, while a postal survey is used in wave 4.

2.3.2.2 Sampling frame

The Child Benefit Register was used as the sampling frame for the GUI study. The Child Benefit Register contains information on Child Benefit payments in Ireland. Child Benefit is paid each month for all children under 16 years (regardless of income) to the person who cares for the child, and must be claimed within six months of the child being born or within six months of the family coming to reside in Ireland. Therefore, the Child Benefit Register is considered an up-to-date and comprehensive database of children residing in Ireland[96]. Non-married subgroups were oversampled

to ensure that there was an adequate absolute number of respondents in these groups. In addition, to ensure non-national infants and their families were adequately represented, a supplementary sample of 700 non-national families were selected (after selection of the main sample).

2.3.2.3 Weighting the data

Survey data needs to be re-weighted because a simple random sample method is rarely used when conducting survey based studies. Therefore, GUI data was re-weighted to ensure the sample was representative of the national sample of infants aged less than one year, and who were on the Child Benefit Register in the 2008 calendar year.

Re-weighting the data allows for the recruited sample to become more comparable with the population of interest, compensating for imbalances in the structure of the recruited sample. Such imbalances may occur due to the lack of availability of an optimal sampling frame, or differential response patterns within subgroups of the population under study[96]. Furthermore, if you fail to re-weight the survey data, estimated standard errors will likely be underestimated, and possibly lead to results that seem to be statistically significant, when in fact, they are not[97].

The GUI data weighting was constructed by adjusting the distribution of the sample to known population figures using Irish Census data and the Child Benefit Register by ensuring that the distribution of individuals in the completed sample matched a set of control totals for the population[96].

2.4 Main Exposure and Outcome Variables

2.4.1 Exposures

2.4.1.1 Preeclampsia

In Swedish data studies (chapters 6, 7 and 8), data on preeclampsia were obtained from the Medical Birth Register, and classified according to the Swedish version of International Classification of Disease (ICD-8) (through 1986), ICD-9 (1987-1996) and ICD-10 (from 1997 onwards)[16].

In the GUI study (chapter 9), data on preeclampsia were self-reported through a questionnaire-based face-to-face interview. The mother was asked the following question: "Were there any of the following complications with the pregnancy?" and instructed to tick all that apply from a list of complications. This included "raised blood pressure and protein in the urine (Preeclampsia)". If the mother ticked this box, then a diagnosis of preeclampsia was assumed.

2.4.1.2 Preeclampsia and Small for Gestational Age

Preeclampsia and small for gestational age (SGA) were combined (i.e. SGA baby exposed to preeclampsia) as a crude proxy for preeclampsia with placental dysfunction. In Swedish data studies, SGA was classified according to the Swedish weight-based fetal growth standard (defined as birthweight <2 standard deviations below the mean of the sex-specific and gestational age distributions)[98] (Chapters 6 and 7).

In the GUI study (chapter 9), SGA was based on maternal-reporting of child's birthweight, gestational age and sex, and defined as birthweight <10th percentile for gestational age and sex of child.

2.4.2 Outcomes

2.4.2.1 Autism Spectrum Disorder

In Swedish data studies (chapters 6 and 8), data on ASD were obtained from the National Patient Register, and classified according to ICD-9, available since 1987 and ICD-10, available since 1997[19].

2.4.2.2 Attention Deficit Hyperactivity Disorder

In Swedish data studies (chapters 7 and 8), data on ADHD were obtained from the National Patient Register and Prescribed Drug Register. A diagnosis of ADHD was determined in one of two ways:

1. If a diagnosis of ADHD was present in the National Patient Register, classified according to ICD-10, available since 1997[88].

2. If the subject was in receipt of ADHD medication in the Prescribed Drug Register, available since 2005. ADHD medication data was classified according to Anatomical Therapeutic Chemical classification system, and included amphetamine (N06BA01), dexamphetamine (N06BA02), psychostimulants methyphenidate (N06BA04) and noradrenergic reuptake inhibitor atomoxetine (N06BA09)[88].

2.4.2.3 Ages and Stages Questionnaire

In the GUI study (chapter 9), data on child development were collected using the Ages and Stages Questionnaire (ASQ). The ASQ was maternal-reported when infants were 9-months, and contains 30 items relating to five developmental domains: communication, gross motor, fine motor, problem solving, and personal/social issues[99].

2.4.2.4 Strengths and Difficulties Questionnaire

In the GUI study (chapter 9), data on behavioural outcomes were collected using the Strengths and Difficulties Questionnaire (SDQ). The SDQ was maternal-reported when infants were 3 years, 5 years, and 7-8 years, and consists of a 25-item questionnaire with five subscales: emotional, conduct, hyperactivity, peer problems and prosocial behaviours[100].

2.5 Statistical modelling

2.5.1 Cox Proportional Hazards Regression Analysis

Cox proportional hazards regression analysis or Cox regression for short is a form of survival analysis whereby time to an event is taken into consideration when investigating longitudinal associations. Individuals within a cohort can enter a study at different times, for example, admission to hospital or date of birth. They are then followed-up until they experience an outcome of interest, they are censored (when individuals are no longer followed-up for reasons other than experiencing the outcome of interest), or the study period ends. Possible reasons for censoring may include death, emigration, or loss-to-follow-up.

Chapters 6, 7 and 8 of this thesis employs Cox regression to examine the association between preeclampsia and ASD and ADHD because children were born and diagnosed at different time points, therefore can enter and exit the study at different times. Results from a Cox regression model can be used to estimate a hazard ratio (HR), which can be interpreted in the same way as a relative risk (RR). The following equation is used to estimate a Cox model:

$$Log(h(t))=Log(h_0(t))+\beta_1 X_1 \ldots + \beta_p X_p$$

where $h(t)$ is the hazard function at time t, $h_0(t)$ is the baseline hazard at time t, and β_1 to β_p is the estimated increase in the risk of the outcome, per unit increase in the value of the exposure variables X_1 to X_p, (where $X_1 = 1$ in the exposed group, and $X_1 = 0$ in the unexposed group)[101].

Assumptions of Cox regression

- Proportional hazards assumption: where the ratio of hazards in exposed compared to unexposed group remains constant over time.
- Non-informative censoring: the reason individuals are being censored is not related to the exposure or outcome of interest.

2.5.2 Sibling-Matched Study

Sibling-matched analysis can be conducted using a stratified Cox regression model, commonly stratified according to families, while also taking different lengths of follow-up time into account. The model assumes family-specific baseline hazards, along with a constant effect of exposure across families on the hazard ratio scale[102]. In an attempt to control for familial lifestyle factors and genetics shared by siblings, this thesis uses sibling-matched analysis in chapters 6 and 7. The analysis includes full and half siblings on the maternal side consisting of a separate stratum for each family, matched on maternal ID. While each family has its own baseline probability of the outcome (ASD/ADHD), reflecting their shared genetic and social factors, the exposure groups (i.e. preeclampsia v non-exposure to preeclampsia) are made within the family, estimating the probability of ASD/ADHD within the family[51].

While conducting a sibling-matched analysis allows to control, at least in part, for shared genetic and familial factors, this analysis only accounts for shared factors that

remain constant between pregnancies. It is possible that some factors may change between pregnancies, and siblings would have a different exposure status. Therefore, these would not be accounted for in the sibling-matched analysis.

2.5.3 Binary Logistic Regression

Binary logistic regression is commonly used when the outcome (dependent variable) has two categories (e.g. dead/alive, pregnant/not pregnant). This method can be used to investigate the relationship between one binary outcome variable and one or more exposures (independent variables). It is commonly used to determine the odds ratio (OR) of an outcome for a one-unit change in the exposure variable. The odds of an event occurring are estimated by transforming the odds using the natural log *(ln)* of the probability of an event. The following equation is used to estimate a logistic regression model:

$$ln(p/1\text{-}p)=\beta_0 + \beta_1 X_1$$

where p is the probability of an event occurring, $1\text{-}p$ is the probability of the event not occurring, and $p/1\text{-}p$ is the odds of the event occurring, β_0 is the intercept (value of y when x=0), and β_1 is the estimated increase in the odds of the outcome, per unit increase in the value of the exposure variable X_1 (i.e. exposure variable association with the outcome).

Chapter 9 includes binary logistic regression to examine the association between preeclampsia and pass/fail of ASQ domains, and preeclampsia and normal/abnormal SDQ domains. It was not possible to use Cox regression analysis in the GUI study as time to event data was not available. Children entered and exited the study at similar times (i.e. exposure status was measured at baseline (9 months), and children were followed-up at age 3 years, 5 years and 7-8 years to measure their outcome status).

Therefore, as information on when the outcome of interest occurred was not available, logistic regression was deemed an appropriate analysis.

Assumptions of binary logistic regression

- The outcome variable must be binary (i.e. two categories).
- P(Y=1) is the probability of the event occurring, therefore it is necessary that the outcome variable is coded accordingly (i.e. 0=does not have the outcome, 1=has the outcome).
- Large sample sizes are required because maximum likelihood estimates (which is how logistic regression models are fit) are less powerful than ordinary least squares (e.g. linear regression). Some statisticians recommend a minimum sample size of 100, and minimum observation to predictor ratio of 10:1).
- Observations are independent (i.e. can only be in one group)[103, 104].

2.5.4 Linear Regression

Linear regression is commonly used when the outcome of interest is continuous. This method uses least-squares to calculate the best-fitting line for the observed data, and estimates the regression coefficients for the associated change in the outcome variable for a given value or change in an exposure/predictor variable. In other words, linear regression models the average change in an outcome variable for a given change in an exposure variable. Chapter 9 uses linear regression as part of a sensitivity analysis to estimate the effect of preeclampsia on both maternal-reported and teacher-reported SDQ score on a continuous scale. By applying linear regression, the average change in SDQ score for a one-unit change in the categorical preeclampsia variable (i.e. from

non-exposed=0 to exposed=1) was estimated[105]. The following equation is used in a linear regression:

$$y=\beta_0 + \beta_1 X_1 + \beta_2 X_2 \ldots + \beta_p X_p + error$$

where y is the outcome variable, β_0 the intercept, β_1 is the partial regression coefficient or unknown constant for the exposure variable X_1, and β_1 and β_2 are partial regression coefficients for covariates/potential confounders $X_{2 \ldots} X_p$.

Assumptions of linear regression

- The outcome variable must be continuous.
- The relationship between exposure and outcome variable should be linear.
- Data should be normally distributed.
- Multi-collinearity should be kept to a minimum. (Multi-collinearity occurs when the independent variables are too highly correlated with each other, or are measuring similar things).
- Homoscedasticity (equal variance): the variance of errors/residuals are similar across the values of the independent variables.
- Observations are independent of one another.

2.5.5 Linear Spline Multilevel Modelling

In longitudinal studies, measurements are sometimes repeated on the same subjects over time creating a two level hierarchy with measurement repetitions or occasions at one level and subjects at another level. As outlined above, an assumption of linear regression is that observations are independent of one another. Therefore, repeated measurements of the same subjects over time will violate this assumption. Furthermore, if multiple measures are included in the same model, the problem of

multi-collinearity can arise due to strong correlations between measurements from the same individual[106]. Therefore, more complex methods are needed to overcome these issues.

Multilevel models are one approach that can be used. Multilevel models take non-independence of repeated measures on the same individual into account, therefore addressing the issue of multi-collinearity[106, 107]. The multilevel approach can also estimate trajectories for all participants regardless of the number of their measurements. Therefore, all individuals with at least one observation can contribute to the model. In addition to this, multilevel modelling takes non-linearity in the trajectory into account. This is an important function of multilevel modelling as associations are not always linear, although it is an assumption of the standard linear regression[108].

As SDQ was measured at three time points (ages 3, 5 and 7-8 years), chapter 9 of this thesis employs linear spline multilevel modelling to model trajectories for preeclampsia-SDQ score and preeclampsia+SGA-SDQ score (on a continuous scale). We used mixed effects models (i.e. containing both fixed effects and random effects), with random effects at two levels: measurement occasion and individual. The linear splines were joined at knot points placed at age 5 and 7-8 years, allowing trajectories to vary between each knot point and each individual.

These models estimate the average intercept and average slope between each knot point (representing the fixed effects), as well as the individual-specific trajectories (representing the random effects), since random effects allow each individual to have different intercepts and slopes[106]. For example, in single level regression models, the average trajectory for the total cohort is modelled. Conversely, multilevel models, not only model the average trajectory, but also individual trajectories with random

intercepts and slopes (i.e. the multilevel model allows each individual to have their own unique baseline SDQ score and differing rates of change)[106]. The following equation is used in a multilevel model:

$$y_{ij}=\beta_0 + u_{0}j+\beta 1\ x\ (age)_{ij} + u_{1j}\ \ x\ (age)_{ij} + e_{0ij}$$

where y_{ij} is the SDQ score for individual $_j$ at time $_i$, β_0 and β_1 represent the fixed estimates for the average intercept and slope, u_0j and u_{1j} represent the random estimates (i.e. the deviation from the average intercept and average slope for individual $_j$) and e_{0ij} is the occasion level residual.

2.5.6 Handling of Missing Data

The best possible method of handling missing data is to prevent the problem in the first instance with meticulous planning of the study and collecting the data carefully. However, this is not always possible and missing data can be an issue even in well-designed studies. This can potentially lead to reduced statistical power, reduced representativeness of the sample, erroneous estimates, and invalid conclusions[109]. Several methods have been proposed to handle missing data, all of which are accompanied by their own strengths and limitations. For example, complete case analysis (also known as listwise deletion) is a simple and most common approach to handling missing data which involves omitting observations with the missing data and analysing the remaining data. This method assumes that data are missing completely at random (MCAR), for example due to faulty equipment, or data being destroyed in a fire. However, if the assumption of MCAR is violated (which is sometimes seen as an unreasonable assumption for many studies), it may introduce bias, while statistical power is also reduced by omitting the observations with missing data[109]. Another method of handling missing data is to use a method known as 'last observation carried

forward.' This method replaces missing values with the last observed value from the same individual, however may lead to biased estimates[109]. A more sophisticated method of handling missing data includes multiple imputation. This method substitutes the missing values with a set of plausible values and produces different versions of the missing data. It begins by predicting the missing data using existing data from other variables. The missing values are then replaced with the predicted values, creating multiple imputed datasets. This method may be more difficult to comprehend than other methods however, and can require more formal training[109].

In this thesis, where a categorical variable had missing data, an extra category was added for the missing values, and included in the various analyses by means of an indicator variable. This method has been proposed for missing confounder data in etiologic research[110]. It ensures all observations are included in the analysis, and if the proportion of missing data are small, it may not have a large impact on effect estimates[111]. However, while this is a relatively simple method to handle missing data, it may introduce bias in non-randomised studies[110]. In addition to the indicator variable method, sensitivity analyses were also conducted when data was not available for specific time periods. For example, in chapter 7, the study population was restricted to 2001-2010 as a sensitivity analysis as outpatient data on ADHD was not available prior to 2001.

2.6 Bias, Confounding, and Chance in Epidemiology

One of the central concerns of epidemiology is to identify causal associations between an exposure and outcome. While it is not possible to make definitive causal claims in observational studies, if researchers can account for external factors that may be leading to an observed association, it may provide evidence for a potential causal

relationship. Therefore, it is important to try to rule out other factors that could be at play that would potentially explain the observed association. Such factors may include bias, confounding and chance. By attempting to account for these factors, which can take place in the design and/or analysis phase of the research study, the potential for a causal-claim may be improved[112].

2.6.1 Bias

Bias refers to systematic error resulting from methodological imperfection. It is independent of study size, and can occur during the design phase of an epidemiological study, resulting in a conclusion, which is different from the truth. Therefore, when investigating the association between an exposure and an outcome, it is important to take steps that would prevent any systematic error being built into the study design, while it is also important to consider the presence of bias when interpreting results[112]. Two common types of bias include selection bias and information bias. Selection bias refers to differences in characteristics between those who participate in a study and those who do not (i.e. the people included in the study are meaningfully different than all who were eligible). For example, mothers and children in a cohort study examining the association between preeclampsia and neurodevelopmental outcomes in offspring may be more likely to drop-out of the study if their child experiences the outcome of interest, potentially biasing results towards the null[113].

Information bias is caused by measurement errors in the ascertainment of the exposure, outcome or potential confounders[112]. The three main types of information bias include

1. Recall bias: This occurs when cases may remember their exposure status differently to controls. For example, in a case-control study, mothers of children, who are aware

of the study hypothesis, whose children have a neurodevelopmental outcome may remember past exposures more accurately than mothers of children without the outcome of interest, potentially overestimating the effect size.

2. Interviewer bias: This occurs when the researcher questions cases and controls differently about their past exposures. For example, in a case-control study, the researcher may ask probing questions about a diagnosis of preeclampsia (or other HDP) to the mothers of children with a neurodevelopmental disorder, potentially overestimating the effect size.

3. Misclassification bias: This occurs when participants in a study are assigned to the wrong exposure or outcome category. Misclassification bias can be differential or non-differential. Differential misclassification is when errors in measurement are only in one direction (i.e. the probability of being misclassified differs between groups in a study)[114]. For example, if only cases are incorrectly assigned to the exposed group, this can potentially lead to an overestimate of the effect size[112]. In comparison, non-differential misclassification occurs when errors in assignment to a group occurs in more than one direction (i.e. the probability of individuals being misclassified is equal across all groups in the study)[114]. For example, the proportion of cases and controls incorrectly assigned to the exposure group are similar, potentially leading to an underestimate of the effect size[112].

2.6.2 Confounding

Confounding is one of the most important issues in epidemiology when attempting to establish a potential causal relationship. Confounding refers to the mixing of the effect on an outcome with the effect of another factor that is associated with the exposure, resulting in a distortion of the true relationship[115].

The three general criteria for a confounder are as follows:

1. Confounders should be associated with the outcome of interest.

2. Confounders should be associated with, but independent of, the exposure of interest.

3. Confounders should not be caused by the exposure (i.e. not on the causal pathway) or the outcome.

There are several ways to control for confounding, and these can take place in the design and/or analysis phase of a research study. During the design phase, three common methods of controlling for confounding include randomisation, restriction and matching. Randomisation takes place in randomised controlled trials. It reduces the potential for confounding by generating groups that are comparable with respect to known and unknown confounding factors. Restriction eliminates variation in a confounder. For example, restricting the study sample to children of mothers who do not smoke to avoid confounding due to maternal smoking. Matching is conducted in case-control studies and involves selecting a comparison group that is forced to resemble cases within a study with respect to one or more potential confounders.

During the analysis phase of a study, stratification and multivariate methods can be used to control for potential confounding factors. Stratification evaluates the exposure-outcome association within each stratum of the confounder. For example, results may be stratified by gestational age. Multivariate analyses, based on statistical modelling, can handle large numbers of confounders simultaneously, and is the main method for controlling for potential confounders used in this thesis. However, when a potential confounder has not been measured or accounted for (possibly because it is unknown or cannot be measured), it can lead to what is known as residual confounding. As it is not possible to rule out the presence of residual confounding in observational research, it is therefore important to take into account when interpreting results[(115)].

2.6.3 Chance

In comparison to bias, chance is caused by random error and can lead to imprecise results. When assessing the role of chance in a study, we are examining how likely it is that the results are a true finding. While random error can occur in all epidemiological studies, it is likely reduced by increasing the study's sample size (i.e. errors from chance will cancel each other out in the long run).

Statistical methods can be used to avoid reporting associations that may be occurring due to chance. Confidence intervals reflect the amount of random error that is present in the sample, and contains a range of likely values of the point estimate, with a specified level of confidence. The level of confidence calculated can vary, for example some researchers use 90% or 99%, however the most commonly used level is 95%. The way to interpret 95% confidence intervals is that if samples of the same size were repeatedly drawn from a population, and their 95% confidence intervals were calculated, then 95% of the confidence intervals would be expected to include the true value of the association[116]. Therefore, if the 95% confidence interval does not include a null association, it is said to be "statistically significant," and unlikely to have occurred by chance.

The following chapters in this thesis outline the studies examining the association between HDP (in particular, preeclampsia) and neurodevelopmental disorders in offspring, including ASD, ADHD, child development and behavioural outcomes. This is achieved by reviewing existing literature, and conducting a range of robust analyses using Swedish National Registry data, and data from a nationally representative study of children living in Ireland.

Chapter 3: ASSOCIATION OF HYPERTENSIVE DISORDERS OF PREGNANCY AND NEURODEVELOPMENTAL DISORDERS IN OFFSPRING: A SYSTEMATIC REVIEW AND META-ANALYSIS PROTOCOL

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Published in: *BMJ Open (2017) (**Appendix 26**)*

3.1 Abstract

Introduction: Hypertensive disorders of pregnancy (HDP), that is chronic hypertension, gestational hypertension, preeclampsia (de novo or superimposed on chronic hypertension) and white coat hypertension, affect up to 10% of pregnancies. HDP-exposure has been linked to an increased likelihood of autism spectrum disorder (ASD), attention deficit/hyperactivity disorder (ADHD), and other neurodevelopmental disorders in children. However, findings are inconsistent and a clear consensus on the impact of HDPs on the likelihood of neurodevelopmental disorders is needed. Therefore, we aim to synthesise the published literature on the relationship between HDPs and the risk of neurodevelopmental disorders in the form of a systematic review and meta-analysis.

Methods and analysis: We will include cohort, case-control and cross-sectional studies in which diagnosis of a HDP was reported and neurodevelopmental disorders were the outcome of interest based on a pre-prepared protocol. A systematic search of PubMed, CINAHL, EMBASE, PsycINFO and Web of Science will be conducted in accordance with a detailed search strategy. Two authors will independently review the titles and abstracts of all studies, perform data extraction using a standardised data collection form, and assess study quality using a bias classification tool. Meta-analyses will be performed to calculate overall pooled estimates using the generic inverse variance method. This systematic review will be reported according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.

Ethics: This proposed systematic review and meta-analysis is based on published data, therefore, does not require ethics approval.

Registration: PROSPERO (registration number: CRD42017068258).

3.2 Introduction

Hypertensive disorders of pregnancy (HDP) are the most common complications of pregnancy estimated to affecting up to 10% of all pregnancies[117, 118]. HDP are classified into four categories, as recommended by the International Society for the Study of Hypertension in Pregnancy (ISSHP)[119]: "chronic hypertension", "gestational hypertension", "preeclampsia – de novo or superimposed on chronic hypertension" and "white coat hypertension". While HDP are not fully understood, risk factors include advanced maternal age and elevated body mass index, both of which are increasingly common in modern society[120]. HDP are associated with multiple pathophysiological changes including reduced placental blood flow, maternal inflammation, and oxidative stress[6]. These can potentially alter fetal developmental trajectories, which may increase the risk of long-term vascular, cognitive, and psychiatric sequelae in the offspring[71, 72, 120, 121].

Neurodevelopmental disorders including autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) are a group of conditions with onset during the developmental period, and may lead to impairments in personal, social, academic, or occupational functioning[23, 122]. Though these disorders have a strong genetic basis[29, 123], there is increasing evidence suggesting that environmental risk factors during prenatal development may also play a role[10, 13, 29, 30, 39, 41, 124]. In support of this, a population-based study conducted on a Swedish population estimated that the environmental contribution of ASD is estimated to be between 17-50%[28, 29]. Furthermore, recent work demonstrated focal patches of abnormal laminar architecture and laminar disorganization in the prefrontal and temporal cortices of children with ASD suggesting there may be alterations in brain development at prenatal stages, as cortical lamination is on-going during the second trimester of

pregnancy[125, 126]. Moreover there is some evidence for alterations in brain structural and vascular anatomy[81] and reduced cognitive functioning[127] in offspring of pregnancies complicated by preeclampsia pregnancies, highlighting the need to determine the impact of HDP-exposure on the risk of adverse neurodevelopmental outcomes in the children.

Early identification and intervention

There is a growing consensus that early identification and intervention are key to improving long-term neurodevelopmental outcomes[128, 129]. Previously published work has indicated that early behavioural intervention if commenced before 30 months old, can lead to improvements in cognitive and adaptive behaviour among individuals with ASD[130, 131]. Despite this increasing recognition for surveillance, the average age of ASD-diagnosis, for example, remains at approximately 4-5 years, meaning the window for early intervention has closed[128, 132, 133]. However, research suggests that a stable diagnosis can be made as young as two years, allowing earlier access to specialised services[134]. Therefore, by examining the potential impact of HDP on neurodevelopment in offspring, it can inform the need for increased paediatric surveillance of infants who have been exposed to HDP. This in turn could allow for early intervention which may aid improvement of neurodevelopmental outcome[128, 130, 131, 135].

Rationale for current systematic review

Evidence suggests that HDP may lead to an increased likelihood of ASD, ADHD as well as other neurodevelopmental disorders in children[30, 136, 137]. Conversely other studies have reported no associations[124, 136], highlighting the need for further study in

this area. Therefore, the aim of this systematic review and meta-analysis is to summarise the available evidence examining the association between HDP, and subsequent likelihood of neurodevelopmental disorders in exposed children, and if possible to identify an overall pooled estimate of association. The systematic review is based on the following requirements:

Population: Pregnant women and their children

Intervention/Exposure: HDP

Comparison: No HDP

Outcomes: *Primary outcome 1:* ASD

Primary outcome 2: ADHD

Secondary outcomes: Neurodevelopmental and other behavioural or cognitive outcomes

Objective

To conduct a systematic review and meta-analysis to examine the association between HDP and neurodevelopmental disorders in the offspring.

3.3 Methods and Design

The systematic review and meta-analysis follows the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines[138].

Criteria for considering studies for the review

Inclusion criteria

► We will include cohort, case-control or cross-sectional studies in which a diagnosis of HDP was reported and neurodevelopmental disorders are the outcome of interest.

► Examining the association between HDP and neurodevelopmental disorders must be part of the main objective of the study. (This includes studies that aimed to look at other perinatal risk factors in addition to HDP).

► Data must be from an original study, and HDP may be confirmed through medical records or doctor-diagnosed self-reporting.

► We will include studies published in English only, including all years from inception of the electronic databases until June 2017.

► Peer-reviewed literature only will be included.

► Neurodevelopmental and other behavioural or cognitive outcomes will be the focus of this review. Motor disorders have been included in the search strategy to capture studies that have included these outcomes.

Exclusion criteria

► Studies which are not in English.

► Studies where the participants are not human.

► Case reports, case-series, letters, commentaries, notes, editorials and conference abstracts.

Search strategy for identifying relevant studies

Bibliographic database searches

1. One reviewer (GMM) will conduct a systematic search of the literature in the following electronic databases: PubMed, CINAHL, EMBASE, PsycINFO and Web of Science. A detailed search strategy has been compiled and these terms will be searched according to the principles of Boolean Logic (AND, OR, NOT) and using Medical Subject Headings (MeSH). For example, ("Preeclampsia" OR "gestational

hypertension") AND ("autism spectrum disorder" OR "attention deficit/hyperactivity disorder" OR "neurodevelopmental disorder"). (**Appendix 1**).

2. Searches of the electronic databases will be supplemented by hand-searching the reference lists of included studies for further potentially eligible studies.

Selection of studies for inclusion in the review

Titles and abstracts of studies retrieved from each database search will be stored and managed in Endnote reference manager©. Two review authors (GMM, ASK) will independently review the titles and abstracts of all studies. Full texts will be obtained where necessary to screen for eligibility in the systematic review and meta-analysis in accordance with the pre-defined inclusion/exclusion criteria. Where consensus on eligibility cannot be achieved, a third review author (GWOK) will be involved in the discussion.

Data extraction and management

Using a standardised data collection form, two reviewers (GMM, GWOK) will independently extract data from the eligible studies including the author and year of publication, study design, definition of exposure and outcome used, sample size, confounders adjusted for (if any) as well as crude and adjusted estimates. Discrepancies will be resolved by a third reviewer (ASK) if necessary.

Appraisal of the quality of included studies

Quality assessment of the included studies will be conducted by two reviewers (GMM, PMK) independently and agreed upon subsequently using an appropriate quality assessment tool. Discrepancies will be resolved by a third reviewer (ASK) if

necessary. A bias classification tool described in detail elsewhere will be used[139]. In summary, this tool uses a checklist to assess common features of the six types of bias most often associated with observational studies (selection, exposure, outcome, analytic, attrition and confounding). Study bias is then classified as minimal, low, moderate, high or not reported for each of the six types of bias and an overall likelihood of bias based on the total of the six types of bias will be measured and reported. For example, selection bias will be minimised if the sample was taken from a 'consecutive unselected population', while conversely a study with high selection bias will arise if sample selection is ambiguous and the sample is not likely representative. (**Appendix 2**).

Data synthesis including assessment of heterogeneity

Where the data allow, meta-analyses will be performed to calculate overall pooled estimates of the relationship between combined HDP, preeclampsia and other categories of HDP, and different disorders of neurodevelopment. Both crude and adjusted results will be displayed where possible using the generic inverse variance method (GIVM). Adjustment will be based on the definition outlined in each identified study. A fixed-effects model will be used where heterogeneity is low (I^2 value of less than 50%), and a random-effects model where heterogeneity is high (I^2 value of 50% or more) according to the Cochrane Handbook criteria[140].

We will also perform the following subgroup/sensitivity analyses where the data allow, using RevMan 5.3:

1) According to study design (cohort vs case-control vs cross-sectional).
2) According to studies that report estimates for the association between preeclampsia and other HDP, and each neurodevelopmental disorder.

3) According to location (for example: Europe vs United States).
4) According to income level of country (low/middle/high).
5) According to study quality (minimal/low vs moderate/high).
6) According to measurement of exposure and outcome data (self-reported vs medical records based on varying clinical coding systems).

Publication bias will be assessed using a funnel plot provided at least 10 or more studies are included in the meta-analysis. The trim and fill method will also be used to identify and correct for funnel plot asymmetry arising from publication bias[141]. Where any other subgroup/sensitivity analyses are identified in the process of the meta-analysis, such as analyses to explore potential high heterogeneity or publication bias, these will be clearly labelled as post-hoc analyses.

Presenting and reporting the results

A flow diagram will be included to outline the step-by-step study selection process, and a rationale provided for excluded studies. The characteristics and quality assessment of the included studies will be presented in tables. Pooled estimates will be presented using forest plots. Where a study is eligible for inclusion in the systematic review but does not provide adequate data to include in a meta-analysis, we will contact the corresponding authors in an attempt to obtain raw data where appropriate. If raw data cannot be obtained, the findings will be included individually in a separate table.

3.4 Conclusion

The systematic review and meta-analysis will summarise existing literature examining the association between HDP and different disorders of neurodevelopment based on

this pre-prepared protocol. By identifying the possible contributors to adverse neurodevelopmental outcomes, it may lead to early identification and intervention. Therefore, by examining potential etiologies of neurodevelopmental disorders, it may inform the need for greater paediatric surveillance of HDP-exposed infants to allow early intervention, which may aid improvement of neurodevelopmental outcome[128, 130, 131, 135].

Potential limitations: It is anticipated that publication bias may pose as a limitation for this review. Studies that show an effect have an increased likelihood of being published, as well as being published in English. Due to limited resources, the systematic review search will be confined to studies published in the English language only, potentially resulting in publication bias as well as relevant indexed studies being overlooked. If possible, a funnel plot will be used to assess the presence of publication bias.

Furthermore, the presence of confounding is a major concern in observational studies. Potential confounders may include infant sex, family's socio-economic status, ethnicity, maternal age, parity, maternal smoking and alcohol consumption, (during pregnancy or during the preconception period) and maternal mental illness, while preterm delivery could potentially play a confounding or mediating role. As mentioned above, our meta-analyses will display both crude and adjusted results where possible using the GIVM, basing adjustment on the definition outlined in each identified study.

Ethics: Given that this is a protocol for a systematic review and based on published data, there is no requirement for ethics approval.

Chapter 4: ASSOCIATION OF HYPERTENSIVE DISORDERS OF PREGNANCY AND NEURODEVELOPMENTAL DISORDERS IN OFFSPRING: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Published in: *JAMA Psychiatry (2018) (**Appendix 26**)*

4.1 Abstract

Importance: While research suggests an association between hypertensive disorders of pregnancy (HDP) and autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD) and other neurodevelopmental disorders in offspring, there is a lack of consensus. Given that HDP is one of the most common complication of pregnancy, it is important to examine its impact on neurodevelopmental outcome.

Objective: Synthesise the published literature on the relationship between HDP and risk of neurodevelopmental disorders in the offspring in the form of a systematic review and meta-analysis.

Data sources: Based on a pre-prepared protocol, a systematic search of PubMed, CINAHL, EMBASE, PsycINFO and Web of Science was performed from inception through June 7, 2017, supplemented by hand-searching reference lists.

Study Selection: Two reviewers independently reviewed titles, abstracts, and full-text articles. English-language, cohort and case-control studies were included in which HDP and neurodevelopmental disorders were reported.

Data Extraction and Synthesis: Data extraction and quality appraisal was performed independently by two reviewers. MOOSE Guidelines were followed throughout.

Main Outcome(s) and Measure(s): Random effects meta-analyses estimated HDP-ASD and HDP-ADHD pooled-odds ratios. Standalone estimates were reported for all other neurodevelopmental disorders.

Results: Of 1,166 studies identified, 61 articles met inclusion criteria. Twenty studies reported estimates for ASD. Eleven of these (including 777,518 participants) reported adjusted estimates, with pooled adjusted odds ratio of 1.35 (95% CI: 1.11, 1.64). Ten studies reported estimates for ADHD. Six of these (including 1,395,605 participants) reported adjusted estimates, pooled adjusted odds ratio 1.29 (95% CI: 1.22, 1.36).

Subgroup analyses according to type of exposure (i.e. preeclampsia or other HDP), showed no statistically significant differences for ASD or ADHD. Thirty-one studies met inclusion criteria for all other neurodevelopmental disorders. Individual estimates were reported for these.

Conclusions and Relevance: Exposure to HDP is associated with an increase in the likelihood of ASD and ADHD. If these findings are causal, they highlight the potential need for greater paediatric surveillance of infants exposed to HDP to allow early intervention which may improve neurodevelopmental outcome.

4.2 Introduction

The International Society for the Study of Hypertension in Pregnancy (ISSHP) classify hypertensive disorders of pregnancy (HDP) into the following categories: "chronic hypertension" (essential/secondary), "white-coat hypertension", "masked hypertension", "transient gestational hypertension", "gestational hypertension" and "preeclampsia" (de novo/superimposed on chronic hypertension)[2]. HDP affect up to 10% of all pregnancies, therefore are among the most common prenatal complications[117, 118]. HDP create adverse *in utero* conditions, which can alter fetal development, and may increase the risk of long-term vascular, cognitive, and psychiatric sequelae in the offspring[71, 72, 120, 121].

Neurodevelopmental disorders, including autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD), are a group of conditions with onset during the developmental period that can impair personal, social, academic, or occupational functioning[23, 122]. It is widely accepted that genetics plays a key role[29, 123] with familial co-aggregation implying shared genetic risk factors[142], however environmental factors may contribute to their etiology[143-145]. A study conducted using Swedish National Registries estimated that environmental contribution of ASD is between 17-50%[28, 29] highlighting the importance of identifying the environmental factors that contribute to the risk of neurodevelopmental disorders in the offspring.

Overall, epidemiological evidence examining the association between HDP and neurodevelopmental disorders remains largely inconsistent[10, 22, 30, 39, 45, 136], and residual or unmeasured confounding is of particular concern in the literature[33, 34, 42, 47-49]. Given the increasing prevalence of HDP, partially due to rising levels of obesity, metabolic syndrome and advanced maternal age[118, 120], collating the existing evidence of the impact of HDP on neurodevelopmental outcome is timely. The objective of this

study was to synthesise the available published literature on the relationship between HDP and the risk of neurodevelopmental disorders in the offspring in the form of a systematic review and meta-analysis.

4.3 Methods

The systematic review was based on the following requirements:

Population: Pregnant women and their children

Intervention/Exposure: HDP

Comparison: No HDP

Outcomes: *Primary outcome 1:* ASD

Primary outcome 2: ADHD

Secondary outcomes: Neurodevelopmental and other behavioural or cognitive outcomes

Data sources and search strategy

The protocol for this systematic review and meta-analysis was registered on PROSPERO, the international prospective register of systematic reviews (CRD42017068258) and was subsequently published[83]. In accordance with Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines[138], one reviewer (GMM) conducted a systematic literature search in the following electronic databases: PubMed, CINAHL, EMBASE, PsycINFO and Web of Science from inception through June 7, 2017. Search terms relating to HDP, ASD, ADHD and other neurodevelopmental disorders were combined according to the principles of Boolean Logic (AND, OR, NOT) and using Medical Subject Headings (MeSH). For example, ("Preeclampsia" OR "gestational hypertension") AND ("autism spectrum disorder"

OR “attention-deficit/hyperactivity disorder” OR “neurodevelopmental disorder”). (**Appendix 1**). Results were limited to English language studies, conducted on humans. No restrictions were placed on publication date, location of study or age of participants. Searches of the electronic databases were supplemented by hand-searching the reference lists of included studies for further potentially eligible studies, and contact with authors was made when a conference proceeding only was located. A post-hoc search of PubMed was also conducted adding the keywords “perinatal complication” OR “prenatal complication” OR “obstetric* complication” to the search strategy.

Study Selection

Two review authors (GMM, ASK) independently reviewed the titles and abstracts of all studies, obtaining full texts where necessary. Where consensus on eligibility could not be achieved, a third review author (GWOK) was involved in the discussion. Eligibility criteria for inclusion in the systematic review included:

► Cohort, case-control or cross-sectional studies in which a diagnosis of HDP was reported and neurodevelopmental disorders were the outcome of interest.

► The association between HDP and neurodevelopmental disorders were part of the main objective of the study. (This includes studies that aimed to look at other perinatal risk factors in addition to HDP).

► Data were from an original study and HDP confirmed through medical records/doctor-diagnosed self-reporting.

► Peer-reviewed literature only.

► Neurodevelopmental and other behavioural or cognitive outcomes only. (Motor disorders were included in the search strategy to capture studies that include these outcomes).

Data extraction

Two reviewers (GMM, GWOK) independently extracted data from eligible studies using a standardised data collection form. Information extracted included author, year of publication, study design, definition of exposure and outcome used, sample size, confounders adjusted for (if any) and crude and adjusted estimates. Any discrepancies were resolved by consensus with a third reviewer (ASK). Authors of two studies were contacted for further information, with a reply received from one.

Bias and quality assessment

Publication bias was evaluated by visually assessing a funnel plot and Egger's test for asymmetry of the funnel plot, where 10 or more studies were included in the meta-analysis[146]. Quality assessment of included studies was conducted by two reviewers (GMM, MM) independently using an appropriate quality assessment tool, and agreed upon subsequently. Any discrepancies were resolved by a third reviewer (ASK). A bias classification tool[139], consisting of a checklist to assess six types of bias most often associated with observational studies (selection, exposure, outcome, confounding, analytic and attrition) was used. Study bias was classified as minimal, low, moderate, high or not reported for each type of bias. An overall likelihood of bias based on the total of the six types of bias was then measured and reported. For example, selection bias was minimised if the sample was taken from a 'consecutive unselected population', while conversely selection bias was categorised as high if

'sample selection was ambiguous and the sample not likely representative'. (**Appendix 2**).

Statistical analysis

Data were analysed using Review Manager 5.3 and Eggers test was conducted in Stata/MP 14.2. Random-effects meta-analyses were performed to calculate overall pooled estimates of the relationship between combined HDP, preeclampsia only, and other HDP, and the outcomes of ASD and ADHD, using the generic inverse variance method (GIVM). GIVM was used to allow studies that do not report raw data to be included in the meta-analyses[140]. Partially adjusted estimates, as a result of matching, were included as crude results and studies that adjusted for confounders in the analysis phase were included as adjusted results. Forest plots were used to display crude and adjusted estimates, with adjustment based on the definition outlined in each identified study.

Subgroup/sensitivity analysis: The following subgroup/sensitivity analysis were decided *a priori:* according to type of HDP (preeclampsia and other HDP), study design, location, income level of country, study quality and measurement of exposure and outcome data. A post-hoc subgroup analysis was performed according to length of follow-up.

4.4 Results

Search results

The original search produced 796 unique results after removal of duplicates (**Figure 4.1**). Of these, 33 full text articles were reviewed following screening of titles and abstracts. Eleven studies were excluded because they did not meet the inclusion

criteria. Reasons for exclusion are outlined in **Figure 4.1**. This resulted in 22 papers. After reviewing reference lists, 38 additional studies were identified. One additional ASD paper was subsequently published and included in the review. A total of 61 papers were included in the systematic review: 20 for ASD (8 identified from original search, 11 from reference lists and 1 subsequently published), 10 for ADHD (4 identified from original search and 6 from reference lists), and 31 for other neurodevelopmental outcomes (10 identified from original search and 21 from reference lists).

Characteristics of studies included in the systematic review

A summary of included studies for ASD and ADHD is available in **Appendix 3** and **Appendix 4**. A summary of studies that report on other neurodevelopmental outcomes, (including cognitive functioning/developmental delay, behavioural outcomes and intellectual disability) along with main findings, is available in **Appendix 5**.

Results of the meta-analyses

ASD: Primary analysis: A total of 20 studies were identified in which a diagnosis of HDP was reported and ASD was the outcome of interest[9-15, 21, 30-36, 49, 50, 62, 147, 148]. The prevalence of HDP among ASD cohort studies ranged from 1.3-9.1%, (mean: 6.2%, median: 6.9% and interquartile range (IQR): 5.3%). Twenty-three estimates from 19 unique studies included crude estimates[9-15, 21, 30-36, 49, 50, 147, 148] and 13 estimates from 11 unique studies included adjusted estimates[9, 10, 13-15, 21, 30, 31, 35, 36, 147] and were included in the meta-analysis[9, 10, 13-15, 21, 30, 31, 35, 36, 147]. **Figure 4.2** displays crude and partially adjusted estimates (as a result of matching), producing a pooled

odds ratio (OR) of 1.41 (95% CI: 1.22, 1.64). A subgroup analysis, examining a preeclampsia-ASD and other HDP-ASD relationship separately, resulted in an OR of 1.37 (95% CI: 1.07, 1.75), and 1.43 (95% CI: 1.17, 1.73) respectively. (Test for subgroup differences: 0.80).

Adjusted estimates reduced the overall HDP-ASD estimate to 1.35 (95% CI: 1.11, 1.64) (**Figure 4.2**). Subgroup analysis examining the preeclampsia-ASD relationship resulted in an OR of 1.50 (95% CI: 1.26, 1.78), while the relationship between other HDP-ASD produced a non-significant OR of 1.25 (95% CI: 0.90, 1.73). However, test for subgroup differences does not indicate a statistically significant difference, p=0.33.

ADHD: Primary analysis: Ten studies were identified in which a diagnosis of HDP was reported and ADHD was the outcome of interest[22, 39-42, 44-48]. The prevalence of HDP among ADHD cohort studies ranged from 0.13-20.8%, (mean: 7.8%, median: 5.5%, IQR: 12.7%). Twelve estimates from nine unique studies included crude results examining the HDP-ADHD relationship[22, 39-42, 44, 45, 47, 48] and eight estimates from six unique studies included adjusted estimates[22, 39-41, 45, 46]. (**Figure 4.3**). Crude pooled estimates produced an OR of 1.32 (95% CI: 1.20, 1.45). In subgroup analysis examining the preeclampsia-ADHD relationship only, the OR was 1.31 (95% CI: 1.19, 1.44), and the OR for the relationship between other HDP and ADHD was 1.62 (95% CI: 1.07, 2.47), (p=0.33).

Adjusted estimates remain relatively unchanged (**Figure 4.3**). (Overall pooled OR: 1.29 (95% CI: 1.22, 1.36)). Results of the subgroup analysis examining the preeclampsia-ADHD relationship (OR: 1.28, 95% CI: 1.22, 1.36), and other HDP-ADHD relationship (OR: 1.70, 95% CI: 1.06, 2.72) were not significantly different, (p=0.24).

Other neurodevelopmental outcomes: Due to varying outcome measures, assessment methods and summary scales used, it was not appropriate to pool results of these studies. Therefore, standalone estimates were reported for 31 unique studies (plus seven studies that were also included as ASD or ADHD outcomes) examining the relationship between HDP and neurodevelopmental, cognitive or behavioural outcomes. A summary of the main findings of these studies is available in **Appendix 5**. Overall, results were largely inconsistent, however, there are few patterns of association. For example, six-out-of-ten studies suggest a positive association between preeclampsia and cognitive impairment within specific populations[57, 58, 60, 61, 69, 149], while four-out-of-five studies suggest a potential link between HDP and intellectual disability[36, 62, 63, 150].

Bias and heterogeneity

Visual inspection of the funnel plot, including adjusted studies only, did not indicate publication bias (**Appendix 6**) (Eggers test: p=0.43). There was moderate heterogeneity for ASD (I^2 = 63%) and low heterogeneity for ADHD (I^2 = 0%) based on adjusted estimates. Heterogeneity among ASD studies was possibly due to differences in confounder adjustments as heterogeneity reduced to 0% when studies that adjusted for maternal age, maternal smoking and parity/birth order were analysed separately (**Appendix 7**). The majority of studies were classified as 'low' or 'moderate' risk of bias (**Appendices 8-10**).

Subgroup/sensitivity analysis: (ASD)

Table 4.1 shows pooled estimates for all studies that reported crude estimates, adjusted estimates, and adjusted estimates according to category of HDP for both ASD and ADHD. Results of the following subgroup analysis are also outlined in **Table 4.1**:

Study design: There were six case-control studies[10, 13-15, 35, 147], (seven estimates), which resulted in a pooled OR=1.47 (1.18, 1.84). Five cohort studies[9, 21, 30, 31, 36], (six estimates) provided an overall non-significant OR=1.26 (0.93, 1.70) (p=0.41).

Location: There were six studies (seven adjusted estimates) from North America[9, 10, 13, 30, 31, 147] OR=1.39 (1.09, 1.77), four studies from Europe[14, 15, 21, 35] (five estimates) OR=1.53 (1.26, 1.87) and one study from Australia[36], OR=0.64 (0.43, 0.95) (p=0.0005).

Income level of country: All ASD studies were conducted in high-income countries.

Study quality: Fourteen studies (16 estimates) were assessed as being minimal/low risk of bias[10, 13-15, 21, 30, 31, 33-36, 49, 50, 148], resulting in a significant OR=1.39 (1.17, 1.65). Five studies (seven estimates) assessed as being moderate/high risk of bias[9, 11, 12, 32, 147], resulted in a non-significant OR=1.18 (0.81, 1.74) (p=0.46).

Exposure measurement: Four studies relying on self-reported measurements of HDP[9, 10, 13, 21] produced five adjusted estimates, resulting in a pooled OR=1.54 (1.07, 2.22). Pooled OR observed among the seven studies (with eight estimates) that relied on medical records for confirmation of HDP[14, 15, 30, 31, 35, 36, 147] was 1.27 (0.99, 1.64) (p=0.39).

Outcome measurement: Two studies (with three adjusted estimates) used maternal-reporting of ASD[9, 21] and produced an OR=1.32 (0.91, 1.91). However, individual point estimates for these studies ranged from 0.96 to 2.10. Pooled results were similar amongst the nine studies (ten estimates) that used medical records to determine ASD

status in the offspring[10, 13-15, 30, 31, 35, 36, 147], producing an OR=1.37 (1.07, 1.75) (p=0.86).

Length of follow up: Five studies had a potential 2-7 years of follow-up[10, 13, 21, 30, 147] with a pooled OR=1.71 (1.23, 2.38) and six studies (eight estimates) had a potential 2-21 years follow-up[9, 14, 15, 31, 35, 36] with a pooled OR=1.22 (0.98, 1.52) (p=0.09).

Subgroup/sensitivity analysis: (ADHD)

Study design: Three case-control studies[39, 40, 46] (with four adjusted estimates) were identified and pooled OR=1.34 (1.25, 1.43), while three cohort studies[22, 41, 45] (four adjusted estimates) resulted in a pooled OR=1.21 (1.10, 1.32) (p=0.08).

Location: Two North American studies[40, 41], two European studies[22, 45] (three estimates) and two 'other' location studies[39, 46] (three estimates) were identified. Results were similar for all subgroups: OR=1.27 (1.13, 1.43), OR=1.26 (1.06, 1.49) and OR=1.27 (0.95, 1.70) respectively, (p=0.99).

Income level of country: Five studies (seven adjusted estimates) were conducted in high-income countries[22, 39-41, 45] compared to one study conducted in an upper-middle income country[46]. Results of a sensitivity analysis, including results from high-income countries only, did not change the pooled results, OR=1.29 (1.22, 1.36).

Study quality: Seven studies (nine estimates) were assessed as being of minimal/low risk of bias[22, 39, 40, 45, 46]. The pooled estimate for these studies was a significant OR=1.29 (1.22, 1.36). Pooled estimate for the three studies (four estimates) assessed as being moderate/high risk of bias[42, 47, 48] was a non-significant OR=0.95 (0.32, 2.76) (p=0.57).

Exposure measurement: Two studies used self-reporting of HDP status[22, 46], resulting in a pooled OR=1.70 (1.06, 2.72), while pooled results of four studies (six estimates)

using medical records to obtain exposure status[39-41, 45], resulted in a pooled estimate of 1.28 (1.22, 1.36) (p=0.24)

Outcome measurement: Five studies (seven adjusted estimates) used medical records to measure ADHD status in the offspring[39-41, 45, 46], compared to one study that used maternal-reporting[22]. However, results of a sensitivity analysis (including medical records only) did not change pooled results, OR=1.28 (1.22, 1.36).

4.5 Discussion

The aim of this systematic review was to synthesise the published literature on the relationship between HDP and the risk of neurodevelopmental disorders in the offspring. This has yielded three principal findings. First, our adjusted pooled results indicate that exposure to HDP is associated with a 35% increased odds of ASD when compared to those unexposed. Results of a subgroup analysis, examining a preeclampsia-ASD relationship in isolation provided an OR of 1.50, while the other HDP-ASD relationship was non-significant with an OR of 1.25. Although subgroup analysis may suggest that the type of HDP may play a role in determining the impact on neurodevelopmental outcome, subgroup differences were not statistically significant.

Second, adjusted pooled results suggest that offspring exposed to HDP are 30% more likely to have ADHD compared to those unexposed. Examining a preeclampsia-ADHD relationship in isolation did not change the estimate, while the odds of ADHD was associated with a 70% increase in relation to other HDP. This subgroup difference was not statistically significant however.

These reported effect sizes are similar to other obstetric risk factors for ASD. For example, caesarean section and advancing maternal age (>35 years) are associated

with a 23-26% and 30% increased odds of ASD respectively[20, 151, 152], while breech presentation and Apgar score <7 may increase the risk of ADHD by 14% and 30%[137]. Third, literature examining the relationship between HDP and other neurodevelopmental, cognitive or behavioural outcomes remain inconsistent. (**Appendix 5**). Some patterns of association were observed between preeclampsia and cognitive impairment when confined to specific populations such as growth restricted, preterm and low birthweight babies[57, 58, 60, 61, 69, 149]. Similarly, the epidemiological evidence examined is suggestive of a potential link between HDP and intellectual disability[36, 62, 63, 150]. However, methodological differences between studies, particularly differences in population and outcome assessment methods, may partially explain the overall lack of consistent findings.

While this study is suggestive of a link between HDP and neurodevelopmental disorders in the offspring, it is difficult to rule out the possibility that antihypertensive medication during pregnancy may be associated with adverse effects in the offspring[153]. However, several potential mechanisms have been proposed in attempts to explain the relationship between HDP and neurodevelopmental outcome. For example, placental dysfunction, associated with HDP, may result in reduced placental perfusion and oxidative stress[70]. In turn, suboptimal nutrient and oxygen availability for the fetus, due to placental insufficiency, may affect the developing brain, increasing the risk of a poor neurodevelopmental outcome[13, 30, 71, 72].

Maternal inflammation may also play a key role. Results of a population-based study in Finland, with data on over one million pregnancies, have shown that inflammatory biomarker, C-reactive protein (CRP) associated with preeclampsia, is significantly associated with a 43% increased risk of autism in offspring, when maternal CRP levels in the highest quintile were compared to the lowest quintile[78, 79]. Fewer hypotheses

have been put forward addressing the biological mechanisms of ADHD specifically, however, it is also possible that similar mechanisms may be involved[22, 81, 82].

In summary, the literature examining HDP and ASD is suggestive of a small increase in the likelihood of ASD in offspring exposed to HDP[9, 11, 13, 14, 21, 30, 35, 50], however some studies fail to meet statistical significance[10, 12, 14, 15, 31, 49, 148]. In contrast, other studies suggest a protective HDP-ASD association[9, 33, 34, 62, 147], with only two reaching statistical significance[32, 36]. Similarly, the literature alludes to a positive relationship between HDP and ADHD, with some studies indicating significant associations[22, 40-42], and others producing non-significant positive estimates[39, 44-46]. In comparison, two HDP-ADHD studies suggest reduced odds of ADHD in HDP-exposed offspring[47, 48]. Notably however, neither study reaches statistical significance or control for potential confounders.

Strengths and limitations

This systematic review had several strengths. It was based on a pre-prepared protocol and MOOSE Guidelines were followed throughout[83]. It included a comprehensive search of five relevant databases, supplemented by hand-searching the reference lists of included studies for further potentially eligible studies.

However, there are also several limitations, including limitations of the current literature. Results were limited to English-language studies only, potentially leading to relevant, non-English studies being overlooked. While the full search strategy was published along with the protocol, it may have been lacking in keywords such "perinatal complication" OR "prenatal complication" OR "obstetric* complication", as hand-searching the reference lists identified a larger number of relevant studies compared to searching the electronic databases. Therefore, we conducted a post-hoc

search of PubMed, adding these words to the search strategy. While this increased the number of hits retrieved five-fold, identifying more eligible studies than the original search strategy, no new studies were identified in the process.

Sample size calculations are lacking in the literature examining the HDP-ASD and HDP-ADHD relationship and may therefore lack statistical power. For example, five out of twenty ASD-studies[11, 12, 33, 49, 147], and three out of ten ADHD-studies had fewer than ten exposed cases[45, 47, 48].

Validated questionnaires were not always used to obtain data, potentially introducing misclassification bias[9, 10, 13, 21, 22, 46], while varying HDP and ASD/ADHD diagnostic criteria may increase clinical heterogeneity between studies[154].

Finally, residual or unmeasured confounding is of particular concern in observational studies, and therefore possible that important confounding factors were not always considered or available[155]. The vast majority of studies included in our meta-analyses identified potential confounders *a priori* based on previous literature, and only two studies[10, 13] appear to have aided this method with directed acyclic graphs to evaluate and assess suspected confounding[156]. Other studies in our review however, fail to control for confounding or do not provide justification for included confounders. Only one ASD-study[21] and one ADHD-study[40] controlled for a combination of key variables such as maternal age, socio-economic status, ethnic origin and family history of mental illness. Therefore, while an apparent association exists between HDP-ASD, and HDP-ADHD, future research examining the association between HDP and neurodevelopmental outcomes needs to identify a comprehensive set of confounders to assess whether this association is causal or whether it is due to residual or unmeasured confounding. Furthermore, this research focused specifically on ASD, ADHD and other neurodevelopmental, behavioural or cognitive outcomes. Future

research could explore the association between HDP and mental disorders not included in this review to gain a greater understanding of the specificity of effects of HDP.

Conclusion

Our systematic review indicates that exposure to HDP is associated with a small increase in the likelihood of ASD and ADHD. If the observed associations were causal, they highlight the potential need for increased developmental screening of HDP-exposed infants to allow early intervention which may improve neurodevelopmental outcome. However, before more definitive conclusions can be reached, more robust research is needed addressing key limitations in the literature.

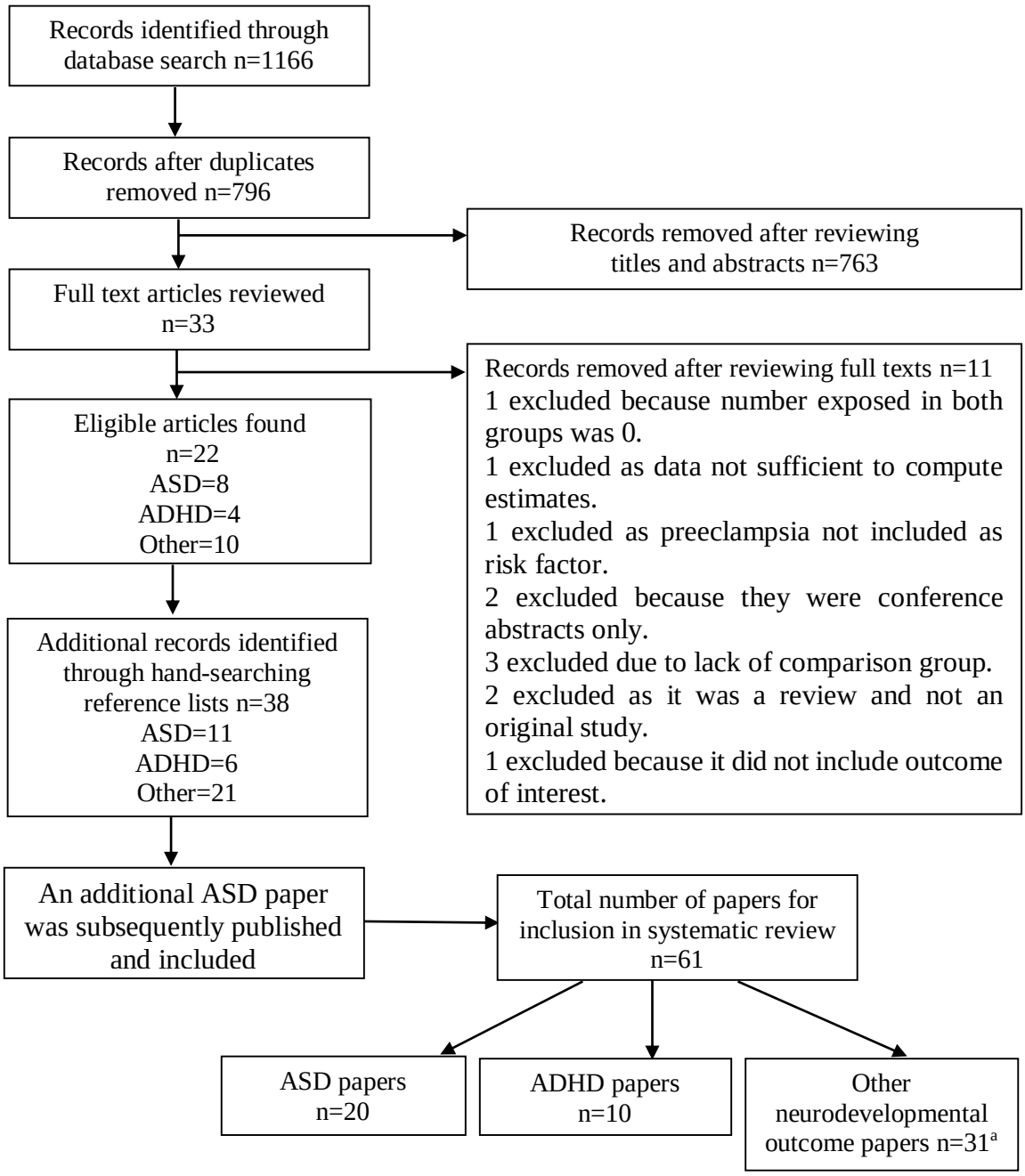
Table 4.1 Subgroup meta-analyses for HDP-ASD and HDP-ADHD

ASD	**Number of studies**	**N**	**Outcomes**	**Odds Ratio**	**95% CI**	**I^2 (%)**	**Test for subgroup differences**
Overall unadjusted	19 (23 estimates)	941285	9331	1.41	(1.22, 1.64)	55	p=0.80[b]
Overall adjusted[a]	11 (13 estimates)	777518	6866	1.35	(1.11, 1.64)	63	p=0.33[b]
Category of HDP[a]							
Preeclampsia	6 (6 estimates)	378991	4254	1.50	(1.26, 1.78)	15	p=0.33
Other HDP	7 (7 estimates)	472268	4621	1.25	(0.90, 1.73)	72	
Study design[a]							
Case-control	6 (7 estimates)	16975	3812	1.47	(1.18, 1.84)	21	p=0.41
Cohort	5 (6 estimates)	760543	3054	1.26	(0.93, 1.70)	78	
Location[a]							
North America	6 (7 estimates)	372527	3555	1.39	(1.09, 1.77)	59	p=0.0005
Europe	4 (5 estimates)	28010	2859	1.53	(1.26, 1.87)	0	
Australia	1 (1 estimate)	376981	452	0.64	(0.43, 0.95)	n/a	
Study quality[c]							
Minimal/low risk of bias	14 (16 estimates)	873772	8041	1.39	(1.17, 1.65)	47	p=0.46
Moderate/high risk of bias	5 (7 estimates)	67513	1263	1.18	(0.81, 1.74)	69	
Exposure measurement[a]							
Self-reported	4 (5 estimates)	81242	2026	1.54	(1.07, 2.22)	68	p=0.39
Medical records	7 (8 estimates)	696276	4840	1.27	(0.99, 1.64)	65	
Outcome measurement[a]							
Maternal-reported	2 (3 estimates)	79543	992	1.32	(0.91, 1.91)	72	p=0.86
Medical records	9 (10 estimates)	697975	5874	1.37	(1.07, 1.75)	63	
Length of follow-up[a]							
Up to seven years	5 (5 estimates)	102838	1823	1.71	(1.23, 2.38)	41	p=0.09
Up to 21 years	6 (8 estimates)	674680	5043	1.22	(0.98, 1.52)	64	
ADHD	**Number of studies**	**N**	**Outcomes**	**OR**	**95% CI**	**I^2 (%)**	**Test for subgroup differences**
Overall unadjusted	9 (12 estimates)	1428209	37635	1.32	(1.20, 1.45)	48	p=0.33[b]
Overall adjusted[a]	6 (8 estimates)	1395605	37128	1.29	(1.22, 1.36)	0	p=0.24[b]
Category of HDP[a]							
Preeclampsia	5 (6 estimates)	1382105	36962	1.28	(1.22, 1.36)	0	p=0.24
Other HDP	2 (2 estimates)	1185896	2489	1.70	(1.06, 2.72)	0	

Study design[a]							
Case-control	3 (4 estimates)	124988	26728	1.34	(1.25, 1.43)	0	p=0.08
Cohort	3 (4 estimates)	1270617	10400	1.21	(1.10, 1.32)	0	
Location[a]							
North America	2 (2 estimates)	166399	21524	1.27	(1.13, 1.43)	70	p=0.99
Europe	2 (3 estimates)	1185896	2489	1.26	(1.06, 1.49)	0	
Other	2 (3 estimates)	43310	13115	1.27	(0.95, 1.70)	0	
Study quality[c]							
Minimal/low risk of bias	7 (9 estimates)	1427617	37365	1.29	(1.22, 1.36)	0	p=0.57
Moderate/high risk of bias	3 (4 estimates)	840	394	0.95	(0.32, 2.76)	67	
Exposure measurement[a]							
Self-reported	2 (2 estimates)	13748	290	1.70	(1.06, 2.72)	0	p=0.24
Medical records	4 (6 estimates)	1381857	36838	1.28	(1.22, 1.36)	0	

Abbreviations: OR=odds ratio. 95% CI=95% confidence interval.
[a]Includes all studies that adjusted for confounders in the analysis phase.
[b]Test for subgroup differences between preeclampsia and other HDP.
[c]Includes all studies

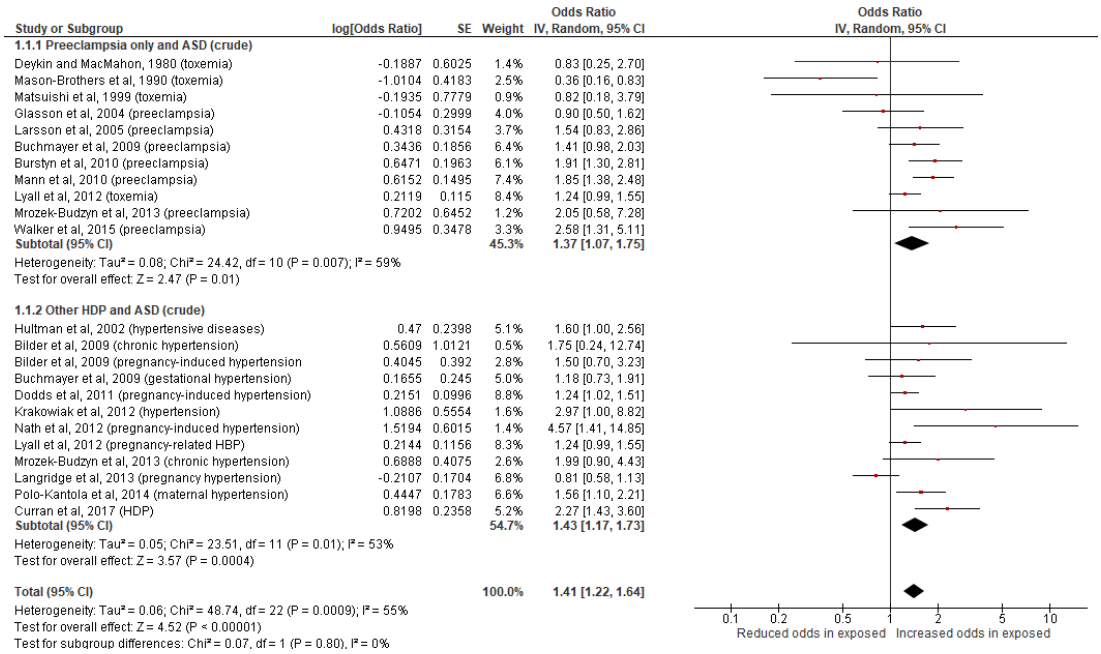
Figure 4-1 Flow diagram of studies selected for inclusion in the systematic review



[a]Plus six studies which are also included in ASD and one in ADHD.

Figure 4-2 Forest plots of the association between HDP and ASD

Crude and partially adjusted estimates



Adjusted estimates

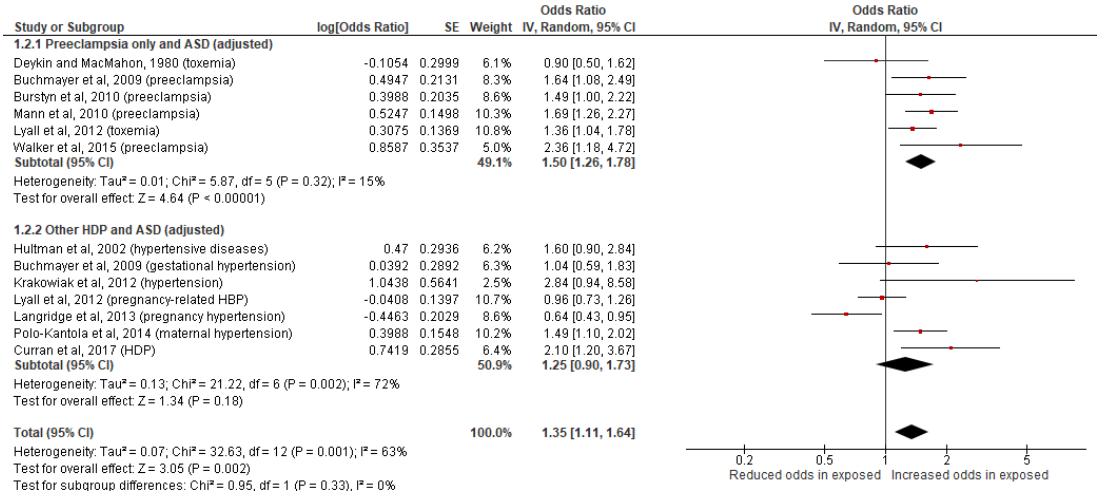
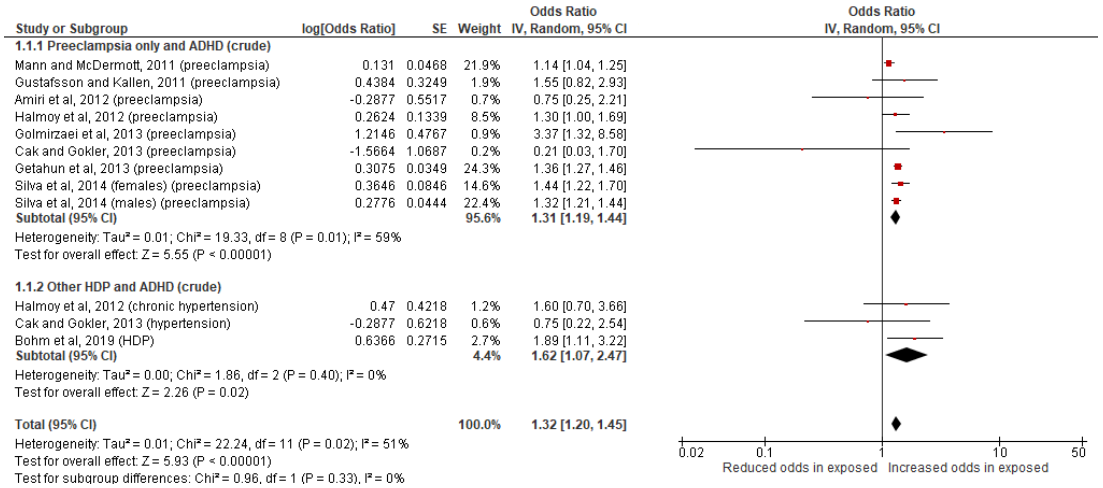
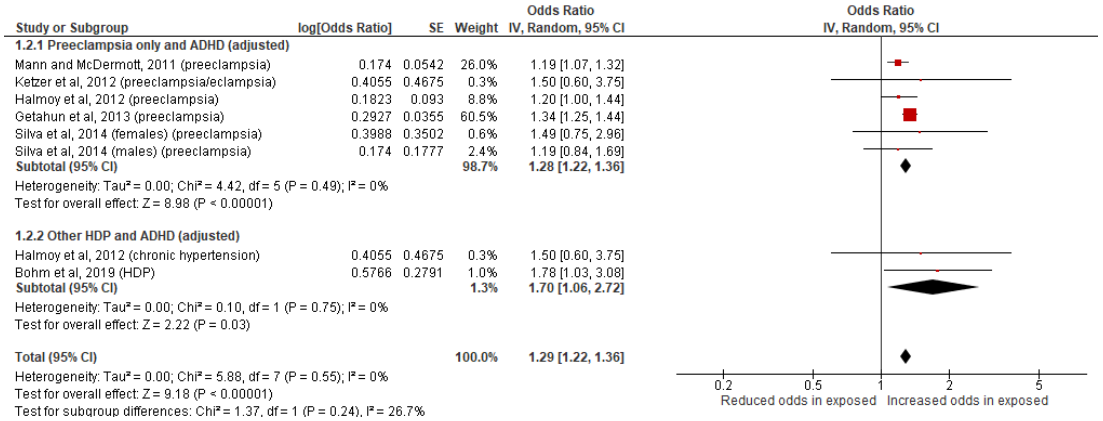


Figure 4-3 Forest plots of the association between HDP and ADHD

Crude and partially adjusted estimates



Adjusted estimates



Chapter 5: A PERSPECTIVE ON PREECLAMPSIA AND NEURODEVELOPMENTAL OUTCOMES IN THE OFFSPRING: DOES MATERNAL INFLAMMATION PLAY A ROLE?

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Published in: *International Journal of Developmental Neuroscience (2018)*

(Appendix 26)

5.1 Abstract

Preeclampsia is a leading cause of maternal death and maternal and perinatal morbidity. Whilst the clinical manifestations of preeclampsia often occur in late pregnancy, the molecular events leading into the onset of this disease are thought to originate in early pregnancy and result in insufficient placentation. Although the causative molecular basis of preeclampsia remains poorly understood, maternal inflammation is recognised as a core clinical feature. While the adverse effects of preeclampsia on maternal and fetal health in pregnancy is well-recognised, the long-term impact of preeclampsia exposure on the risk of autism spectrum disorder (ASD) in exposed offspring is a topic of on-going debate. In particular, a recent systematic review has reported an association between exposure to preeclampsia and increased risk of ASD, however the molecular basis of this association is unknown.

Here we review recent evidence for; 1) maternal inflammation in preeclampsia; 2) epidemiological evidence for alterations in neurodevelopmental outcomes in offspring exposed to preeclampsia; 3) long-term changes in the brains of offspring exposed to preeclampsia; and 4) how maternal inflammation may lead to altered neurodevelopmental outcomes in preeclampsia-exposed offspring. Finally, we discuss the implications of this for the development of future studies in this field.

5.2 Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder, characterised by impairments in social and communication skills, as well as restricted and repetitive patterns of behaviour[25, 157, 158]. ASD is among the most common neurodevelopmental conditions with a prevalence of approximately 1% globally, and 1.5% in developed countries[25, 26].

While there is a general consensus that genetics play the major role in the etiology of ASD[29], the environmental contribution is estimated to be between 17-50%[28, 29]. Therefore, it is important to investigate factors potentially contributing to the likelihood of development of ASD. Several environmental risk factors, including prenatal and perinatal factors have been examined in an attempt to explain the etiology of ASD[25]. In particular, a recent systematic review examining the association between hypertensive disorders of pregnancy (HDP) and neurodevelopmental disorders reported an association between preeclampsia and ASD in exposed offspring[56]. However, the molecular basis of this association is not known. Therefore, the objectives of this paper are to review and provide a perspective on the:

1. Evidence for maternal inflammation in preeclampsia;
2. Epidemiological evidence for alterations in neurodevelopmental outcomes in offspring exposed to preeclampsia;
3. Evidence for long-term changes in the brains of offspring exposed to preeclampsia;
4. Evidence for how maternal inflammation may lead to altered neurodevelopmental outcomes in preeclampsia-exposed offspring.

Hypertensive disorders of pregnancy

Hypertensive disorders of pregnancy (HDP) may be chronic (pre-dating pregnancy or diagnosed before 20 weeks' gestation) or arise *de novo* (either preeclampsia or gestational hypertension). HDP are one of the most common gestational complications affecting 3-10% of all pregnancies and are made up of a collection of hypertensive conditions including pre-existing hypertension (chronic hypertension), gestational hypertension, white coat hypertension and preeclampsia[(2)]. Of these, preeclampsia is one of the leading cause of maternal mortality and morbidity and has recently been redefined by the International Society of Hypertension in Pregnancy (ISSHP) as gestational hypertension (systolic BP $\geq$140 and/or diastolic BP $\geq$90 mmHg) accompanied by one or more of the following new-onset conditions at or after 20 weeks' gestation[(2)]:

1. Proteinuria;
2. Other maternal organ dysfunction, including:
    - Acute kidney injury (creatinine >90umol/L; 1mg/dL)
    - Liver involvement (elevated transaminases e.g. ALT or AST >40 IU/L) with or without right upper quadrant or epigastric abdominal pain)
    - Neurological complications (examples include eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, persistent visual scotomata)
    - Haematological complications (thrombocytopenia – platelet count below 150,000/uL, DIC, hemolysis)
3. Uteroplacental dysfunction (such as fetal growth restriction, abnormal umbilical artery Doppler wave form analysis, or stillbirth).

Previously thought to be simply due to impaired trophoblast invasion followed by the development of the clinical manifestations of the disease, it is now appreciated that the underlying etiology of preeclampsia is far more complex. Beginning with genetic susceptibility, followed by an abnormal immune adaptation to pregnancy, this in turn leads to impaired placentation and perfusion of the intervillous space by oxygenised arterial blood resulting in excessive or deficient placental derived factors in the maternal circulation[6, 159]. The endothelial dysfunction, resulting from placental ischemia and release of placental products which occurs in preeclampsia appears to occur as a result of oxidative stress and is mediated by high levels of free radicals and low levels of antioxidants[160-165]. Vasoactive factors released include soluble fms-like tyrosine kinase-1 (sFlt-1), cytokines, angiotensin II and type 1 receptor autoantibodies [166-170]. These factors target the maternal vascular endothelium giving rise to the maternal syndrome of hypertension, proteinuria, organ and uteroplacental dysfunction which may be followed by acute atherosis in the spiral arteries predisposing to spiral artery thrombosis and placental infarcts[171, 172]. In addition, there is an increasing awareness that preeclampsia leads to a state of exaggerated maternal inflammation[6] meaning that it may be one of the most common causes of maternal inflammation during pregnancy, a recognised risk factor for adverse neurodevelopmental outcomes[173]. Consequently, there has been a growing interest in studying maternal inflammation and subsequently neurodevelopmental outcomes in offspring exposed to preeclampsia.

5.3 Maternal inflammation in preeclampsia: a role for Interleukin-6?

In uncomplicated pregnancies there is a normal systemic inflammatory response in which cytokines promote the infiltration of the spiral arteries by invading trophoblast

cells[73]. This is an important feature of normal placentation and occurs early in pregnancy. However this normal inflammatory response becomes exaggerated in preeclampsia resulting in disruptive activation of monocytes, granulocytes and the endothelium resulting in a state of maternal inflammation[174]. Interestingly many clinical studies have now reported that women with preeclampsia have increased levels of inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-12 and IL-16, which cause structural and functional changes in endothelial cells, promote the formation of endothelin and reduce acetylcholine induced vasodilatation[166, 175-184]. A systematic review and meta-analysis published in 2013 tested the association between preeclampsia and maternal circulating levels of IL-6 (*n* = 425 preeclampsia and *n* = 363 normotensive), IL-10 (*n* = 180 preeclampsia and *n* = 175 normotensive) and TNF-α (*n* = 1015 preeclampsia and *n* = 925 normotensive)[185]. Third trimester maternal circulating levels of IL-6, IL-10 and TNF-α were significantly higher in women with preeclampsia compared to normotensive controls[185]. Subsequently a number of studies have extended and corroborated these findings.

A study by Mihu et al.[186] examined maternal cytokine concentrations between 28 and 41 weeks' gestation in a normal pregnancy group (*n* = 78), a preeclampsia group (*n* = 80), and a non-pregnant control group (*n* = 72) and reported elevations in IL-6 and TNF-α in the preeclampsia group[186]. In support of this, a study by Valencia-Ortega et al.[187] also examined IL-6 levels in age-matched pregnant women with preeclampsia (*n* = 50) and without preeclampsia (*n* = 50). They reported that maternal serum concentrations of IL-6 were significantly higher in late-onset preeclampsia, compared to early-onset preeclampsia or normal pregnancy[187]. Moreover while mid-gestation circulating IL-6 levels were associated with preeclampsia, IL-6 was only

significantly associated with term preeclampsia, suggesting that elevations in IL-6 may be a late stage feature of preeclampsia[188]. This is consistent with a study of women with preeclampsia (n = 208) and normotensive controls (n = 411) which showed that first and second trimester levels of IL-6 were not associated with preterm preeclampsia[189]. Interestingly given that we and others have reported that the stage of pregnancy in which offspring are exposed to maternal inflammation is a key determinant of neurodevelopmental outcomes in exposed offspring[190-195], it is possible that the effects of preeclampsia on offspring neurodevelopmental outcomes may vary depending on the severity and clinical course of the disease.

These findings are supported by animal modelling of the pathophysiological mechanisms that underlie the development of preeclampsia. Specifically, the reduced uterine perfusion pressure (RUPP) rat model of placental ischemia mimics many of the clinical characteristics of preeclampsia. Placental ischaemia generated by reductions in uterine perfusion pressure in pregnant rats increases blood pressure, reduces glomerular filtration rate (GFR), increases sFlt-1 concentrations, elevates production of pro-inflammatory cytokines and reactive oxygen species (ROS) and leads to intrauterine growth restriction (IUGR)[196]. Recent studies in the RUPP model have described an immune imbalance characterised by increased pro-inflammatory $CD4^+$ T cells and pro-inflammatory cytokines in addition to a reduction in regulatory T cells and anti-inflammatory cytokines[197]. Specifically there is substantial evidence of increased serum levels of pro-inflammatory cytokines IL-6[198] and TNF-α[199] in response to placental ischemia in the RUPP model compared to sham controls. A study by Gadonski et al.[198] examined the role of IL-6 in generating preeclampsia-like characteristics by infusing pregnant rats with IL-6 for 5 days resulting in a 2-3 fold increase in serum IL-6 levels. As a result of the increase in circulating IL-6 levels these

rats had elevated mean arterial pressure, reduced renal plasma flow and reduced glomerular filtration rates[198]. Interestingly, these preeclampsia-like characteristics were not evident in virgin rats infused with IL-6[198]. These data indicate that elevations in maternal IL-6 may be part of the maternal inflammatory pathophysiology of preeclampsia.

5.4 The epidemiological evidence for alterations in neurodevelopmental outcomes in offspring exposed to preeclampsia

We recently conducted a systematic review synthesising published, epidemiological evidence examining the association between HDP and neurodevelopmental disorders in the offspring[56]. The primary outcomes included in the review were ASD and attention deficit hyperactivity disorder (ADHD). Secondary outcomes included behavioural outcomes such as Asperger's Syndrome, Pervasive Developmental Disorder - Not Otherwise Specified (PDD-NOS), behavioural difficulties using standardised checklists, as well as cognitive functioning, developmental delay and intellectual disability. In total, 61 papers were included in the review: 20 for ASD (six cohort studies and 14 case-control studies), 10 for ADHD (five cohort studies and five case-control studies) and 31 secondary outcome papers (25 cohort studies and six case-control studies).

Pooled results from this study showed that exposure to HDP (including preeclampsia, gestational hypertension and chronic hypertension) was associated with a 35% increase in the odds of ASD when compared to those unexposed to HDP (OR=1.35; 95% CI: 1.11-1.64)[56]. Subgroup analysis examining preeclampsia alone and ASD increased the odds ratio to 1.50 (95% CI: 1.26-1.78), whereas all other HDP (which may include preeclampsia) were associated with a non-significant increase in the odds

of ASD (OR: 1.25, 95% CI: 0.90-1.73)[56]. (**Table 5.1**). However, it is important to note that the epidemiological evidence in this area is largely inconsistent. For example, some studies suggested that exposure to preeclampsia may be associated with a statistically significant increase in the likelihood of ASD, when compared to unexposed offspring[9, 13, 14, 30], while others proposed a positive other HDP-ASD relationship[11, 21, 35, 50]. Similarly, there are studies that alluded to a positive preeclampsia-ASD relationship[12, 14, 31, 148], and other HDP-ASD relationship[10, 14, 15, 49] but failed to meet statistical significance. Conversely, some older studies are suggestive of a protective association between preeclampsia and ASD[9, 32-34, 147], and other HDP-ASD[9, 36], but only two of these found a statistically significant relationship[32, 36].

Given the non-significant pooled estimate seen with other HDP and ASD, it is difficult to hypothesise whether the type of HDP is an important factor in determining the impact on ASD risk in exposed offspring. The subgroup analysis by Maher et al, 2018[56] reported a statistically significant association between preeclampsia and ASD but a non-significant risk of ASD with other HDP (which may include preeclampsia). This may suggest that the association observed occurs not as a result of exposure to hypertension but as a result of exposure to a mediator of the complex syndrome of preeclampsia such as inflammation. More research is needed on the association between type of HDP and ASD in order to examine whether preeclampsia only or all HDP display a significant association with ASD.

Although the findings show an apparent HDP-ASD relationship, results may need to be interpreted with caution as several limitations were identified among ASD studies. Firstly, misclassification bias could have resulted from a lack of validated questionnaires and maternal reporting of exposure and ASD status when determining

exposure and outcome status of subjects[9, 10, 13, 21]. Secondly, confounding is of particular concern in observational studies due to the lack of randomisation process, potentially leading to spurious findings. The vast majority of studies identified in the systematic review failed to control for a combination of key variables, calling into question the validity of findings. For example, only one study controlled for a combination of key variables such as maternal age, socio-economic status, ethnic origin and maternal depression[21]. Finally, several studies contained small sample sizes, evident in 5 of 20 studies which had fewer than 10 cases of ASD exposed to HDP[11, 12, 33, 49, 147]. However, results of larger studies (>10 exposed cases) that controlled for at least one potential confounder in the analysis phase of the study ranged from an OR of 1.36 to 2.36 for preeclampsia and 0.96 to 2.83 for other HDP (which may have included preeclampsia)[56].

In addition, while the results of the systematic review also suggest an association between preeclampsia and ASD, this association may not be specific to ASD[56]. For example, adjusted pooled results in Maher et al.[56] also proposed that offspring exposed to HDP were 30% more likely to have ADHD compared to those unexposed. Subgroup analyses investigating a preeclampsia-ADHD relationship in isolation did not change this estimate, while the odds of ADHD was associated with a 70% increase in relation to other HDP[56]. Moreover, while the evidence remains inconsistent among secondary outcome studies included in the review, there were some patterns of association between HDP and intellectual disability despite methodological differences between studies[36, 62, 63, 150]. For example, results from Griffith et al. 2011[63] suggested that preeclampsia/eclampsia was associated with a 38% increase in the odds of intellectual disability (95% CI: 1.16, 1.64)[63]. Similarly, the relative risk for an eclampsia-'mental retardation' relationship classified according to ICD coding

was 3.03 in Danish offspring less than 15 years old[62]. Langridge et al.[36] measured intellectual disability using the American Association on Mental Retardation classification system and suggested an association between HDP and moderate intellectual disability in Western Australia (OR: 1.39, 95% CI: 1.25, 1.54)[36]. Lastly, Salonen et al.[150] used a standardised set of tests for mental performance and suggested that HDP was associated with an increased likelihood of 'mental retardation' in children aged 9-10 years in Eastern Finland (RR: 6.1, 95% CI: 1.3, 28.9)[150].

Collectively, the epidemiological evidence points to an apparent relationship between preeclampsia exposure in particular, and ASD risk in exposed offspring. However, the specificity of the effects of preeclampsia on ASD risk, could in fact be associated with poor neurodevelopmental outcome in general as opposed to being specific to ASD[200]. Given the available evidence that preeclampsia and other HDPs may impact neurodevelopmental outcomes[56], there has been an increasing focus on identifying any neuroanatomical alterations in the brain of offspring exposed to preeclampsia.

5.5 Evidence for long-term changes in the brains of offspring exposed to preeclampsia

An increasing body of work has now shown that the brains of women with preeclampsia can undergo structural and functional changes as a result of preeclampsia with the suggestion that this may predispose to developing neurological deficits later in life (for a comprehensive review see Ijomone et al[201]). However, aside from the maternal neurological changes, there is increasing interest in how preeclampsia exposure can lead to long-term changes in brains of exposed offspring.

A recent imaging study has examined regional brain volumes and cerebral vasculature of children aged 7 to 10 years after exposure to preeclampsia[127]. Specifically children that had been exposed to preeclampsia (mean age = 9.79 ± 0.89 years; *n* = 10; 5 male, 5 female) were matched based on age and sex to those born from an uncomplicated pregnancy (mean age = 9.66 ± 1.07 years; *n* = 10; 5 male, 5 female). This cohort then underwent magnetic resonance (MR) imaging to identify any brain structural and vascular anatomic differences. While there were no significant differences in total intracranial brain volume between the control group and children from mothers exposed to preeclampsia, the preeclampsia group had significant larger regional brain volumes in five of twenty-one regions analysed that included the cerebellum, temporal lobe, left amygdala, right amygdala and the brain[127]. It is important to note however that there were no significant differences in gestational age (controls = 39.47 ± 1.38 weeks vs. preeclampsia = 37.16 ± 3.34 weeks), there was a significant difference in birthweight (controls = 3.42 ± 0.36 kg vs. preeclampsia = 2.67 ± 0.79 kg) in this study which may have confounded these results[127]. For example, children born at 37 weeks' gestation were found to have poorer school performance compared to those born at 39-41 weeks' gestation, an effect that was independent of birthweight[202].

Interestingly, however, these alterations in regional brain volumes have also been reported in children with ASD[203, 204]. In particular, the increases in amygdala volume has been reported in a number of studies[205]. In addition, a recent follow up study in this same cohort in the Ratsep et al.[127] study employed diffusion tensor MR imaging (DTI) to examine myelination patterns and white matter connectivity and six brain regions of interest were identified for analysis by tractography (middle occipital gyrus, caudate nucleus and precuneus, cerebellum, superior longitudinal fasciculus, and cingulate gyrus)[206]. They reported increased tract volumes in a number of these brain

regions including the superior longitudinal fasciculus, which is strongly related to language and communication pathways[207]. Interestingly, while the molecular mechanisms underlying these neuroanatomical changes are unknown, a recent study has shown that exposure of fetal cortical neurons to serum of women with established preeclampsia lead to increases in axonal growth and branching[21]. This suggests that preeclampsia exposure may alter neurodevelopmental trajectories, but to our knowledge this causative basis of altered brain volumes in offspring exposed to preeclampsia are currently unknown and these studies require confirmation in larger patient cohorts.

5.6 How might maternal inflammation in preeclampsia alter neurodevelopmental outcome?

Given the epidemiological evidence for an association between preeclampsia and neurodevelopmental outcome, then a key question is what are the mechanisms that mediate this association? Given that maternal inflammation is a core feature of the maternal pathophysiology of preeclampsia[185] and systematic evidence has reported maternal inflammation as a risk factor for ASD[208], it is possible that preeclampsia-induced maternal inflammation is a determinant of fetal neurodevelopmental outcome. Arguably IL-6 is the best characterised mediator of the impacts of maternal inflammation on fetal neurodevelopmental outcome. Animal models of maternal inflammation have shown that maternal administration of the viral mimetic, poly(I:C), lead to elevations in maternal and fetal IL-6 levels, and alter neurobehavioural outcomes in the offspring[195]. Blocking IL-6 signalling through maternal co-administration of anti-IL-6 antibodies with poly(I:C), prevented the poly(I:C)-induced social deficits and transcriptional changes in the brains of exposed

offspring[209]. Interestingly there is also increased IL-6 expression and signalling in the placenta in the poly(I:C) model suggesting that conditions that increase maternal-placental IL-6 signalling may lead to detrimental effects in the fetal brain[210]. This has recently been addressed in an elegant study by Wu et al.[211] who addressed the role of maternal IL-6. The authors crossed *il-6*$^{+/+}$ males with *il-6*$^{-/-}$ females (resulting in a pregnant dam who cannot mount an IL-6 response), and in parallel crossed *il-6*$^{-/-}$ males with *il-6*$^{+/+}$ females (resulting in a pregnant dam who can mount an IL-6 response). Poly(I:C) administration to these pregnant dams led to increases in fetal brain IL-6 levels only in offspring from *il-6*$^{+/+}$ females[211]. Moreover, conditional deletion of the IL-6 receptor in the placental trophoblast prevented the maternal poly(I:C)-induced fetal brain inflammatory response, neuroanatomical changes and anti-social and repetitive/anxiety-like behaviour in exposed offspring[211].

While the majority of these studies have been carried out in rodent models, a recent study in humans reported the association between maternal IL-6 in pregnancy and the structural connectivity of frontolimbic circuitry, which is critical for socioemotional and cognitive development, in 30 infants[77]. Specifically, diffusion tensor imaging revealed that maternal IL-6 levels averaged across pregnancy were inversely associated with fractional anisotropy (a measure of brain connectivity) and offspring cognition at 12 months of age[77]. Other studies have also show that third trimester maternal IL-6 levels, are associated with neonatal functional connectivity and with both fetal heart rate variability and toddler cognitive development[76]. This is in agreement with the report that higher average maternal IL-6 was prospectively associated with larger right amygdala volume and selected stronger bilateral amygdala connectivity[212]. Interestingly, larger newborn right amygdala volume and stronger left amygdala connectivity mediated the association between

higher maternal interleukin-6 concentrations and lower impulse control at 24 months of age[212]. Collectively these data support the premise that preeclampsia-induced alterations in maternal IL-6 and maternal-placental IL-6 signalling may be key determinants of any neuroanatomical and neurobehavioural changes in offspring exposed to preeclampsia-induced maternal inflammation. (**Figure 5.1**).

5.7 Conclusions and future perspectives

Future epidemiological research examining the association between preeclampsia and ASD in particular and neurodevelopmental disorders in general, should address the limitations and gaps in the current literature we have recently discussed[56]. In particular, large population-based cohort studies with valid methods to identify women with HDP and children with ASD are needed. It is important that such studies be able to adjust for key potential confounders such as maternal age, maternal body mass index, socio-economic status factors, family history of mental disorders, and ethnic origin. In addition, such studies should attempt to assess whether observed associations between HDP and ASD is HDP type specific, whether the association is specific to ASD, or ASD and other neurodevelopmental and psychiatric disorders. Whether other pregnancy complications and early life events have effect modification or mediation role in the HDP-ASD association is worth investigating as such analyses may improve our understanding of the association and the potential mechanisms. Moreover an important gap in the literature is the potential impact of antihypertensive medications on any observed association. In other words, is the observed association between preeclampsia and ASD related to the HDP or pharmacological treatments used during pregnancy. This is an important question for future research.

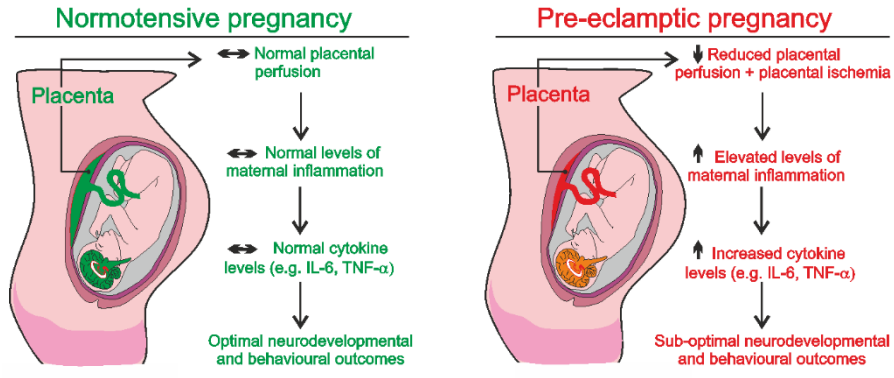
In future work it will also be important to examine neuroanatomical and neurobehavioural outcomes across the life span using both the RUPP model of preeclampsia, and in clinical cohorts. While the longitudinal nature of these studies are challenging in humans, imaging and developmental assessments of adequately powered cohorts of offspring exposed to preeclampsia and appropriate matched controls will be important given recent studies showing changes in the brains of preeclampsia-exposed offspring[127, 206]. Combining this with animal modelling will allow the role of maternal inflammation and in particular IL-6 as mediator of the association to be determined, using elegant approaches reported by Wu et al.[211]. Moreover, given a recent study showing that most significant genetic variants associated with schizophrenia, converge on a developmental trajectory sensitive to events that affect the placental response to *in utero* stressors, including preeclampsia[213], understanding the placental response in preeclampsia and how this may predict or be associated with neurodevelopmental outcomes in preeclampsia-exposed offspring[214], will be important questions for future research.

Table 5.1 Summary of studies examining the association between preeclampsia and ASD

Author	Design	N	Prenatal Stressor	Outcomes	Results	cOR/RR (95% CI)	aOR/RR (95% CI)
Walker et al, 2015[13]	Case-control	867	PE from medical records or maternal self-reporting in telephone interview	ADOS ADI-R	↑ odds ASD*	2.58 (1.31, 5.11)	2.36 (1.18, 4.72)
Mrozek-Budzyn et al, 2013[12]	Case-control	288	PE from medical records or self-reporting	ICD-10	↑ odds ASD	2.05 (0.58, 7.28)	-
Lyall et al, 2012[9]	Cohort	66445	Toxemia self-reported in questionnaire	Maternal-reported	↑ odds ASD*	1.24 (0.99, 1.55)	1.36 (1.04, 1.78)
Burstyn et al, 2010[31]	Cohort	216342	PE from APHP delivery records	ICD-9	↑ odds ASD	1.91 (1.30, 2.81)	1.49 (1.00, 2.22)
Mann et al, 2010[30]	Cohort	87677	PE/eclampsia from billing records for Medicaid-eligible women (ICD-9)	ICD-9 from Medicaid billing records or DDSN	↑ odds ASD*	1.85 (1.38, 2.48)	1.69 (1.26, 2.27)
Buchmayer et al, 2009[14]	Case-control	7296	PE from MBR (ICD-9, ICD-10)	ICD-9, ICD-10	↑ odds ASD*	1.41 (0.98, 2.03)	1.64 (1.08, 2.49)
Larsson et al, 2005[148]	Case-control	18148	PE from MBR	ICD-8 and ICD-10 from PCR	↑ odds ASD	1.54 (0.83, 2.86)	-
Glasson et al, 2004[34]	Case-control	1627	PE (ICD-9)	DSM	↓ odds ASD	0.90 (0.50, 1.62)	-
Eaton et al, 2001[62]	Case-control	103021	Eclampsia from MBR	ICD from PCR	↓ odds ASD	0.82 (NR)	-
Matsuishi et al, 1999[33]	Case-control	232	Toxemia	DSM-III-R	↓ odds ASD	0.82 (0.18, 3.79)	-
Mason-Brothers et al, 1990[32]	Case-control	285	Toxemia from medical records	DSM-III	↓ odds ASD	0.36 (0.16, 0.83)	-
Deykin et al, 1980[147]	Case-control	364	Toxemia from medical records and interview data	≥1 symptoms of impaired relatedness to the environment, stereopathy and impaired language development	↓ odds ASD	0.83 (0.25, 2.70)	0.90 (0.50, 1.62)

*Adjusted result was statistically significant.
Abbreviations: cOR/RR=crude odds ratio/relative risk. aOR/RR=adjusted odds ratio/relative risk. 95% CI=95% confidence interval. PE=preeclampsia. ADOS=Autism Diagnostic Observation Schedule. ADI-R=Autism Diagnostic Interview, Revised. ICD=International Classification of Disease. APHP=Alberta Perinatal Health Program. DDSN=Department of Disabilities and Special Needs, South Carolina. MBR=Medical Birth Register. PCR=Psychiatric Central Register. DSM=Diagnostic and Statistical Manual of Mental Disorders. NR=not reported. DSM-III-R=Diagnostic and Statistical Manual of Mental Disorders–3rd Edition Revised.

Figure 5-1 Schema showing an overview of how preeclampsia may impact neurodevelopmental outcomes in exposed offspring



While physiological levels of maternal inflammation plays a role in normal pregnancy, the decrease in placental perfusion in preeclampsia leads to the increased production of pro-inflammatory cytokines including IL-6 and TNF-α. These cytokines may disrupt placenta signalling and/or cross to the fetal circulation to alter fetal neurodevelopmental trajectories, which may increase the risk of sub-optimal neurodevelopmental outcomes in offspring exposed to preeclampsia.

Chapter 6: ASSOCIATION BETWEEN PREECLAMPSIA AND AUTISM SPECTRUM DISORDER: A POPULATION-BASED AND SIBLING-MATCHED COHORT STUDY

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Published in: *Journal of Child Psychology and Psychiatry (2020) (**Appendix 26**)*

6.1 Abstract

Background: The environmental contribution of autism spectrum disorder (ASD) is approximately 17-50%, highlighting the importance of investigating factors potentially contributing to the likelihood of its development, and of gaining a greater understanding of the pathogenesis surrounding ASD. The objective of this study was to examine the association between preeclampsia and ASD using a population-based cohort study.

Methods: All singleton live births in Sweden from 1982-2010 were included, using data from Swedish National Registers. Exposures of interest included: **1**. Preeclampsia (classified according to ICD-8, ICD-9 and ICD-10) **2.** Preeclampsia and small for gestational age (SGA) combined, used as a proxy for preeclampsia with placental dysfunction. ASD status was based on ICD-9 and ICD-10.

The cohort consisted of 2,842,230 children, with 54,071 cases of ASD. Follow-up began from the child's first birthday and data were censored at first diagnosis of ASD, death, migration or end of study period (31st December 2016). We conducted multivariate Cox proportional hazards regression analysis, adjusting for several perinatal and sociodemographic factors, selected *a priori*. We further controlled for shared genetic and familial confounding using sibling-matched analysis.

Results: In the adjusted Cox proportional hazards regression analysis, preeclampsia was associated with a 25% increase in the likelihood of ASD (Hazard Ratio (HR): 1.25, 95% CI:1.19, 1.30) compared to those unexposed to preeclampsia, while in the sibling-matched analysis the HR was 1.17 (95% CI:1.06, 1.28). The HR for

preeclampsia and SGA combined was 1.66 (95% CI:1.49, 1.85) in the adjusted Cox model and 1.95 (95% CI:1.53, 2.48) in the sibling-matched analysis.

Conclusions: Exposure to preeclampsia or preeclampsia/SGA (i.e. SGA baby exposed to preeclampsia) was associated with ASD. The stronger association with preeclampsia/SGA than preeclampsia alone suggests that placental pathology may be a mechanism for the increased likelihood of ASD.

6.2 Introduction

Autism spectrum disorder (ASD) is characterised by persistent impairments in interpersonal interaction and restricted or repetitive patterns of behaviour[23]. The prevalence of ASD is approximately 1.5%[26, 215], and while genetics play a major role in the development of ASD, the environmental contribution is estimated to be between 17-50%[28, 29]. This highlights the importance of investigating factors contributing to the likelihood of its onset, and potentially facilitate the development of appropriate interventions[74]. Furthermore, while often comorbid with intellectual disability, previous results indicate that risk factors for ASD with and without intellectual disability may differ, and is therefore important to examine ASD according to the presence or absence of intellectual disability[36, 216].

Preeclampsia is one of the leading causes of maternal morbidity and mortality and has recently been redefined by the International Society for the Study of Hypertension in Pregnancy (ISSHP) as new-onset hypertension (blood pressure ≥140/90 mmHg on/after 20 weeks' gestation) accompanied by proteinuria and/or other maternal organ dysfunction and/or uteroplacental dysfunction[2]. Preeclampsia is associated with maternal inflammation, poor placentation and oxidative stress, which may also represent some of the potential etiological pathways in the development of ASD[71, 79, 217].

While there is conflicting evidence regarding a preeclampsia-ASD relationship, pooled estimates from epidemiological research suggest preeclampsia is associated with a 50% increase in odds of ASD[56]. However, several limitations of the existing literature, including residual confounding (for example, family lifestyle factors such as diet), small sample sizes, and poor phenotyping and use of definitions of

hypertensive disorders of pregnancy versus preeclampsia, need to be addressed before more definitive conclusions can be reached.

Therefore, the objective of this study was to examine the association between preeclampsia and ASD (overall, and stratified by ASD with and without intellectual disability), while addressing the key limitations in the literature outlined above.

6.3 Methods

Study Population

The study population consisted of all singleton live births in Sweden from 1982 to 2010 using data from the Swedish Medical Birth Register. The Medical Birth Register was linked to the National Patient Register, Multi-generation Register, Total Population Register and Register of Education using personal identification numbers (PIN) assigned to each Swedish resident, in order to conduct the study.

Similar to previous ASD-studies conducted on this population[19, 29], follow-up began from the child's first birthday (or 1st January 1987, when the ICD-code for ASD became available). Data were censored at first diagnosis of ASD, death, migration or end of study period (i.e. 31st December 2016). This is in contrast to Sandin et al. and Curran et al. who included follow-up data until the end of 2009 and 2011 respectively[19, 29].

Ethical approval was previously obtained from the Stockholm Regional Ethical Review Board (number 2010/1185-31/5), and informed consent was waived by the ethics committee.

Exposures

Preeclampsia: Data on preeclampsia was obtained from the Medical Birth Register. The Medical Birth Register, established in 1973, contains data on over 97% of all births in Sweden, and includes information on prenatal care, delivery, neonatal care and maternal socio-demographic and lifestyle factors[85]. However, since 1982, standardised copies of antenatal, obstetric and pediatric records were used to collect data, while quality data on obesity and smoking status during pregnancy also became available, marking the beginning of our study[16].

A doctor reviews discharge records and notes a diagnosis of preeclampsia at the time of discharge from the hospital using a standard form, containing the definition of preeclampsia, accompanied by an ICD-code and checkbox. These are forwarded to the National Board of Health and Welfare for inclusion in the Birth Register. Preeclampsia is classified according to the Swedish version of ICD-8 (through 1986), ICD-9 (1987-1996) and ICD-10 (from 1997 onwards)[16].

1. Preeclampsia: ICD-8 [code 637]: Gestational hypertension (blood pressure ≥140/90mmHg on/after 20 weeks' gestation), accompanied by proteinuria (≥0.3g/day or ≥1 on a urine dipstick) or edema (positive predictive value (PPV)=50%)[16].

ICD-9 [code 642]: Gestational hypertension accompanied by proteinuria (PPV=96%)[16].

ICD-10 [code O14 or O15]: Gestational hypertension accompanied by proteinuria.

2. Preeclampsia with placental dysfunction: We combined preeclampsia and small for gestational age (SGA) as a proxy for preeclampsia with placental dysfunction, as SGA is closely associated with uteroplacental dysfunction[218]. SGA was classified according to the Swedish weight-based fetal growth standard (defined as birthweight

<2 standard deviations below the mean of the sex-specific and gestational age distributions)[98].

Outcome

Data on ASD and intellectual disability were obtained from the National Patient Register. The National Patient Register contains information on inpatient psychiatric diagnoses from 1973 (obtaining complete national coverage in 1987)[87, 215]. Outpatient data is available in the National Patient Register since 2001[215] (coverage of data from private caregivers is approximately 80%, and public caregivers almost 100%)[87]. ASD is classified according to ICD-9 [code 299], available since 1987 and ICD-10 [code F84], available since 1997 (PPV=94.3%)[19]. Therefore, index persons (IP) who turned one year of age before 1987 began follow-up on 1st January 1987, when an ICD-code for ASD first became available.

As risk factors for ASD with/without intellectual disability may differ[36, 216], we examined ASD overall, and also stratified results by ASD with intellectual disability (defined as IQ<70)[215, 219] and ASD without intellectual disability. For example, if cases of ASD did not receive a diagnosis of intellectual disability throughout the study period, they were considered to have ASD without intellectual disability. (Intellectual disability is classified according to ICD-9 [code 317-319] and ICD-10 [code F70–F79])[216].

Confounding Variables

Confounders were based on previous literature, and limited to the data available in the National Registers. They were examined through the use of a directed acyclic graph to gain a visual representation of the potential confounder pathways. (**Appendix 11**).

We obtained year of birth, infant sex, maternal age, maternal and paternal country of birth, birth order, maternal smoking status, body mass index (BMI) at first antenatal visit, and gestational weight gain from the Medical Birth Register. Similar to a previous ASD study conducted on this population[19], we also controlled for maternal and paternal depression, bipolar disorder, and non-affective psychiatric disorders, obtained from the National Patient Register. Socioeconomic factors including family income and parental level of education were obtained from the Total Population Register and Register of Education. Information on all confounders was available for the entire study period with the exception of parental level of education, available since 1990. (**Appendix 12**).

Statistical Analysis

Data were analysed using Stata/MP 14.2. Multivariate Cox proportional hazards regression analysis was performed to estimate HR and 95% confidence intervals, for preeclampsia; preeclampsia and SGA (i.e. SGA baby exposed to preeclampsia); and preeclampsia without SGA, and likelihood of ASD (overall and with/without intellectual disability). The proportional hazards assumption was assessed graphically and based on Schoenfeld residuals. Partially adjusted models were stratified by year of birth in order to satisfy the proportional hazard assumption (model 1). Fully adjusted models (model 2) controlled for year of birth, infant sex, maternal age, maternal and paternal country of birth, birth order, parental depression, bipolar disorder and non-affective psychiatric disorders, maternal smoking status, BMI at first antenatal visit, gestational weight gain, family income and parental level of education. To account for the possibility of increased diagnosis of ASD in recent years, and due

to a reliance on inpatient psychiatric diagnoses until 2001, we also stratified results by decade of birth.

Sibling-matched analysis: To control for unmeasured confounding factors shared by siblings, including family environment, lifestyle factors such as diet, maternal characteristics, and genetic factors, we conducted a sibling-matched analysis (model 3) using stratified Cox regression. This method is an extension of the paired binomial model, taking into account different lengths of follow-up time. The analysis included full and half siblings on the maternal side consisting of a separate stratum for each family, matched on maternal ID. While each family has its own baseline probability of ASD, reflecting their shared genetic and social factors, the exposure groups (i.e. preeclampsia v non-exposure to preeclampsia) are made within the family, estimating the probability of ASD within the family[51]. We adjusted for the same potential confounders as model 2 with the exception of maternal country of birth as this is the same across sibling pairs.

Post-hoc analysis: We examined the association between SGA-alone and ASD compared to non-exposure to SGA/preeclampsia.

Sensitivity analyses: As the definition of preeclampsia from 1982-1986 does not correspond to later years, and the National Patient Register obtained complete national coverage in 1987, we performed a sensitivity analysis restricting the study population to 1987-2010. In addition, we excluded births after 2006 to ensure each individual was followed-up for a minimum of 10 years.

Classifying preeclampsia into ‘mild’ or ‘severe’ is not recommended in clinical practice. However, preeclampsia may present with or without severe features[2]. As delivery is the only effective cure for preeclampsia, gestational age is often used as a proxy for severity. For example, preeclampsia could be considered severe if delivery

occurred before 34 weeks' gestation[220]. As a result, sensitivity analyses were conducted examining the relationship between preeclampsia-ASD (in those born ≥34 weeks' gestation) and preeclampsia-ASD (in those born <34 weeks' gestation) compared to deliveries at ≥34 weeks' gestation in mothers with no preeclampsia, using the full cohort.

Further sensitivity analyses included 'preeclampsia without chronic hypertension' as the exposure, and 'preeclampsia with chronic hypertension' as the exposure. We examined the association between preeclampsia-ASD excluding those with a family history of mental illness, while we also included caesarean section in a multivariate model. Furthermore, we analysed the relationship between preeclampsia with low/intermediate APGAR score at five minutes. We examined a preeclampsia-ASD relationship by maternal age, in addition to preeclampsia-ASD by BMI group at time of first antenatal visit. Finally, subgroup analyses examined a preeclampsia-ASD relationship by gestational age and gender while controlling for potential confounders. (Gestational age was defined according to ultrasound measurements, or from information of the last menstrual period)[221].

6.4 Results

Descriptive Statistics

Table 6.1: There were 2,941,628 live births recorded in the Swedish Medical Birth Register between 1st January 1982 and 31st December 2010. After exclusions, (figure 1) 2,842,230 children remained in the final cohort. Of these, 1,460,940 (51.4%) were male and 1,381,290 (48.6%) were female. There were 77,600 (2.7%) children exposed to preeclampsia. There were 54,071 (1.9%) cases of ASD with a median age of diagnosis of 14 years. Of these, 2,024 were exposed to preeclampsia.

Association between Preeclampsia and ASD

Table 6.2: In the fully adjusted model (model 2) preeclampsia was associated with a 25% increase in the likelihood of ASD (HR: 1.25, 95% CI: 1.19, 1.30) compared to those unexposed to preeclampsia, and this association was reduced in the sibling-matched analysis (model 3) (HR 1.17, 95% CI: 1.06, 1.28). The HR for preeclampsia and SGA combined was 1.66 (95% CI: 1.49, 1.85) in model 2 and 1.95 (95% CI: 1.53, 2.48) in model 3, and the HR for preeclampsia without SGA was 1.20 (95% CI: 1.14, 1.26) in model 2 and 1.11 (95% CI: 1.01, 1.23) in model 3.

Preeclampsia and ASD with intellectual disability

Table 6.2: Preeclampsia was associated with a 56% increase in the likelihood of ASD with intellectual disability (HR: 1.56, 95% CI: 1.41, 1.73) in model 2 and 32% increase in model 3 (HR: 1.32, 95% CI: 1.07, 1.62). Those exposed to preeclampsia and SGA were nearly 3 times more likely to have ASD with intellectual disability in model 2 (HR: 2.95, 95% CI: 2.40, 3.64), with similar results observed in model 3 (HR: 3.07, 95% CI: 1.97, 4.79). The HR for preeclampsia without SGA was 1.40 (95% CI: 1.24, 1.57) in model 2 and 1.15 (95% CI: 0.91, 1.45) in model 3.

Preeclampsia and ASD without intellectual disability

Table 6.2: The HR for preeclampsia was 1.19 (95% CI: 1.13, 1.25) in model 2, and 1.13 (95% CI: 1.01, 1.26) in model 3. Preeclampsia and SGA were associated with a 42% increase in likelihood of ASD without intellectual disability (HR: 1.42, 95% CI: 1.25, 1.62) in model 2, and 63% in model 3 (HR: 1.63, 95% CI: 1.22, 2.19). The HR for preeclampsia without SGA was 1.17 (95% CI: 1.10, 1.23) in model 2, and 1.10

(95% CI: 0.98, 1.23) in model 3. Stratifying results by decade did not materially change results (**Table 6.3**).

Post-Hoc Analysis

The adjusted HR for an SGA only-ASD relationship was 1.60 (95% CI: 1.53, 1.67), while in the sibling-matched analysis, the HR was 1.82 (95% CI: 1.65, 2.01) (**Table 6.2**).

Sensitivity Analyses

When the study population was restricted to 1987-2010, as association between preeclampsia and ASD was still observed. Similarly, excluding births after 2006 did not materially change results. Fully adjusted results of the sensitivity analysis suggested that preeclampsia exposure in those born at ≥34 weeks' gestational age was associated with an 18% increase in the likelihood of ASD (HR: 1.18, 95% CI: 1.13, 1.24) when compared to those unexposed to preeclampsia, and born at a similar gestational age. The fully adjusted result for preeclampsia in those born at <34 weeks' gestational age (used as a proxy for preeclampsia with severe features) was 2.04 (95% CI: 1.81, 2.30) when compared to non-exposure to preeclampsia in those born at ≥34 weeks' gestation. The HR for a preeclampsia-ASD relationship, excluding those with chronic hypertension, was 1.26; and including those with both preeclampsia and chronic hypertension was a non-significant 0.91. The fully adjusted HR for preeclampsia (excluding those with family history of mental illness) was 1.28, while including caesarean section in the multivariate model resulted in a HR of 1.21. Preeclampsia with a low/intermediate APGAR score at five minutes increased the likelihood of ASD by 30% compared to non-exposure to preeclampsia and

low/intermediate score. Finally, preeclampsia among mothers <20 years of age and mothers with a BMI of <20 was associated with the highest odds of ASD (HR: 1.37 and 1.29 respectively) compared to those of similar maternal age and BMI at first antenatal visit. (See **Appendix 13** and **Appendix 14** for full description of results).

Subgroup Analyses

Adjusted subgroup analysis suggested a statistically significant increase in the likelihood of ASD at all gestational ages when compared to non-exposure to preeclampsia in those born at ≥37 weeks' gestation. When adjusted for potential confounders, exposure to preeclampsia was associated with a 25% increase in the odds of ASD in both male and female offspring. (**Appendix 13** and **Appendix 15**).

6.5 Discussion

This study aimed to examine the association between preeclampsia and ASD (overall and with/without intellectual disability) and has yielded two principal findings. First, exposure to preeclampsia was associated with 25% increased odds of ASD when compared to those unexposed, after controlling for known potential confounders. The sibling-matched analysis allowed us to further control for shared genetic and familial factors and reduced the HR to 1.17. However, when results were stratified by ASD with and without intellectual disability, the HRs were 1.32 and 1.13 respectively. These data are largely in line with a previous systematic review, which suggested that preeclampsia was associated with a 50% increase in the odds of ASD, with individual study estimates ranging from 0.90 to 2.36[56].

Second, as SGA is closely associated with uteroplacental dysfunction[218], we combined preeclampsia and SGA as a crude proxy for preeclampsia with placental

dysfunction. This decision is also in line with the recent guidelines put forward by ISSHP to include uteroplacental dysfunction in the definition of preeclampsia[2].

Being an SGA baby and exposed to preeclampsia was associated with a 95% increased odds of ASD when compared to non-exposure to preeclampsia or SGA. This HR increased to 3.07, when stratified by ASD with intellectual disability, and reduced to 1.63 when stratified by ASD without intellectual disability[222]. This observed preeclampsia and SGA relationship with ASD suggests that impaired placentation may be a common factor increasing the likelihood of ASD. Furthermore, the post-hoc analysis examining SGA-alone and ASD further supports this hypothesised mechanism given the modest effect of preeclampsia on likelihood of ASD compared to that of preeclampsia and SGA combined, or SGA-alone.

The precise biological mechanisms contributing to a preeclampsia-ASD relationship are still unknown however. In a previous study, we demonstrated that exposure of fetal neurons to maternal serum from term preeclampsia altered fetal cortical neuronal growth and branching[21], while treatment of fetal cortical neurons with conditioned media from preeclamptic placentae also had similar effects, suggesting secreted factors may be important[223]. Such factors may include inflammatory cytokines given that preeclampsia is associated with chronic immune activation, leading to a significant increase in the circulation of pro-inflammatory cytokines. Thus, while uncomplicated pregnancies have a normal systemic inflammatory response[73], preeclampsia results in a state of exaggerated maternal inflammation[73, 224]. Therefore, maternal inflammation, a recognised risk factor for poor neurodevelopmental outcome, could act as a mediator between preeclampsia and development of ASD, and the pro-inflammatory cytokine interleukin (IL)-6 may be a leading candidate in this regard[74].

Straughen et al, 2017[225] demonstrated that placental inflammation of any type is associated with an increased likelihood of ASD, while circulating levels of maternal IL-6 have been shown to be inversely associated with brain connectivity and offspring cognition at 12 months of age, as well as short and long-term influences in offspring behaviour in separate studies[76, 77]. This may also partially explain the increased HR when results were stratified by ASD with intellectual disability, as elevated mid-gestational levels of numerous cytokines and chemokines such as GM-CSF, IFN-γ, IL-1α, and IL-6 are associated with ASD with intellectual disability, when compared to mothers of children with either ASD without intellectual disability, developmental delay, or general population controls[222].

In terms of mediation, while very little data exist in humans, a recent study has shown that maternal depressive symptoms are associated with higher maternal inflammation, including IL-6, and this mediated the effect on maternal report of infant negative affect[226], a known risk factor for later adverse neurological outcomes. This may also suggest that preeclampsia-induced elevations in maternal IL-6 may act as a mediator of the preeclampsia-ASD association.

Finally, the role of concurrent exposure to antihypertensive medication in the development of ASD was beyond the scope of this paper, and needs to be explored in future research. This research question could possibly be addressed using animal models such as the reduced uterine perfusion pressure (RUPP) model in rats, which mimics many physiological features of preeclampsia[227], in order to study the impact of antihypertensive medications administered using clinical relevant treatment protocols, on neurobehavioural outcomes in offspring.

Strengths and Limitations

This study had several strengths. It is the largest epidemiological study to investigate the association between preeclampsia and ASD, with data on over 2.8 million births. Information on exposure and outcome status was classified according to ICD-coding, obtained from national registers. Therefore, selection bias and recall bias were not likely an issue. The use of registry data allowed us to control for a wide range of confounding variables, while conducting a sibling-matched analysis allowed us to further control, at least in part, for shared genetic and familial factors.

However, several limitations may also pose a threat to validity of findings. One, each individual in the present study was followed-up until they reached a minimum of six years of age (i.e. those born in 2010 followed-up until 2016). While it is possible that not enough time had lapsed for a diagnosis of ASD to be received by some individuals, excluding births after 2006 to ensure everyone had at least 10 years of follow-up does not materially change results. Two, the prevalence of ASD in the current study was 1.9%, compared to previous ASD studies conducted on this population who had a ~1% prevalence of ASD[19, 29]. However, we included follow-up data until the end of 2016, whereas Sandin et al. and Curran et al.[19, 29] included follow-up data until the end of 2009 and 2011 respectively. This means that each child in the present study was followed-up for 5-7 additional years compared to the two previous studies. If we restrict our follow-up date to 2011, it results in a more comparable prevalence to that of previous studies (~1%). Given that children are often not diagnosed with ASD until they are of school age, it is suspected that the extended follow-up is the reason for the difference in ASD prevalence[228].

Three, severe cases may have been overrepresented in our data due to a reliance on inpatient psychiatric diagnoses until 2001[87]. While results of a sensitivity analysis by

decade of birth (**Table 6.3**) were not significantly different from our main findings, the HR of 4.39 for SGA babies exposed to preeclampsia in children with ASD and intellectual disability born 2000-2010 warrants highlighting, and could possibly reflect an increased awareness of ASD or increased diagnostic specificity in recent decades.

Four, a lack of robust data on gestational hypertension limited our analysis. Results of existing studies suggest a non-significant gestational hypertension-ASD relationship[56]. However, if a gestational hypertension-ASD association existed, this would bias our results towards the null.

Finally, despite controlling for several potential confounders, residual confounding may still be an issue. While this was reduced in the sibling-matched analysis, this method can only adjust for factors constant between pregnancies, therefore we cannot rule out the possibility of unmeasured confounding factors[229].

Conclusion

The apparent preeclampsia/SGA-ASD relationship suggests that placental pathology may be a common factor increasing the likelihood of ASD. Further research is needed to investigate the role that maternal inflammation may play, as well as the potential impact of pharmacological treatments used during pregnancy on likelihood of ASD.

Table 6.1 Perinatal and sociodemographic characteristics related to preeclampsia and ASD among singleton live births in Sweden between 1982 and 2010

	No. (%) of Infants			
Characteristic	**Total Population**		**Preeclampsia**	
Total Population	2842230		77600	(2.7)
ASD	54071	(1.9)	2024	(2.6)
ASD with intellectual disability	8981	(0.3)	388	(0.5)
ASD without intellectual disability	45090	(1.6)	1636	(2.1)
SGA	69355	(2.5)	9761	(12.7)
First-born child	1210413	(42.6)	49756	(64.1)
Sex (male)	1460940	(51.4)	40475	(52.2)
Decade of birth				
1982-1989	773489	(27.2)	19596	(25.3)
1990-1999	1006338	(35.4)	27635	(35.6)
2000-2010	1062403	(37.4)	30369	(39.1)
Maternal age, years				
<20	66946	(2.4)	2393	(3.1)
20-29	1495876	(52.6)	41463	(53.4)
30-39	1210467	(42.6)	31217	(40.2)
≥40	68941	(2.4)	2527	(3.3)
Gestational age, weeks				
<34	32,332	(1.1)	6375	(8.2)
34	17,162	(0.6)	2276	(2.9)
35	29,982	(1.1)	3080	(4.0)
36	60,016	(2.1)	5155	(6.7)
37	141036	(5.0)	8583	(11.1)
38	386963	(13.6)	12516	(16.1)
39	657765	(23.2)	14653	(18.9)
40	799752	(28.2)	13942	(18.0)
>40	712440	(25.1)	10894	(14.1)
5-Minute Apgar score				
0-3 (low)	5530	(0.2)	307	(0.4)
4-6 (intermediate)	20589	(0.7)	1599	(2.1)
7-10 (high)	2772613	(99.1)	74412	(97.5)
Delivery completed by caesarean section	260,650	(9.2)	19,574	(25.2)
Mother's country of birth				
Sweden	2272714	(80.0)	64024	(82.5)
Other Nordic country	85743	(3.0)	2309	(3.0)
Other country	336123	(11.8)	6602	(8.5)
Missing	147650	(5.2)	4665	(6.0)
Father's country of birth				
Sweden	2244697	(79.0)	63454	(81.2)
Other Nordic country	76280	(2.7)	2008	(2.6)
Other country	354182	(12.5)	6909	(8.9)
Missing	167071	(5.9)	5229	(6.7)
Maternal depression				
Never	2473216	(87.0)	66912	(86.3)
Before birth	44440	(1.6)	1355	(1.7)
After birth	177106	(6.2)	4676	(6.0)
Missing	147468	(5.2)	4657	(6.0)
Maternal bipolar disorder				
Never	2669867	(93.9)	72242	(93.1)
Before birth	3527	(0.1)	115	(0.1)
After birth	21368	(0.8)	586	(0.8)

Missing	147468	(5.2)	4657	(6.0)
Maternal nonaffective disorders				
Never	2674249	(94.1)	72359	(93.2)
Before birth	6898	(0.2)	207	(0.3)
After birth	13615	(0.5)	377	(0.5)
Missing	147468	(5.2)	4657	(6.0)
Paternal depression				
Never	2564110	(90.2)	69636	(89.7)
Before birth	24621	(0.9)	698	(0.9)
After birth	106031	(3.7)	2609	(3.4)
Missing	147468	(5.2)	4657	(6.0)
Paternal bipolar disorder				
Never	2679318	(94.3)	72562	(93.5)
Before birth	2661	(0.1)	75	(0.1)
After birth	12783	(0.4)	306	(0.4)
Missing	147468	(5.2)	4657	(6.0)
Paternal nonaffective disorders				
Never	2675845	(94.1)	72458	(93.4)
Before birth	7155	(0.3)	200	(0.2)
After birth	11762	(0.4)	285	(0.4)
Missing	147468	(5.2)	4657	(6.0)
Smoking at first antenatal visit				
No	2186399	(76.9)	63720	(82.1)
1-9 cigarettes/day	300389	(10.6)	5886	(7.6)
≥10 cigarettes/day	165015	(5.8)	2849	(3.7)
Missing	190427	(6.7)	5145	(6.6)
BMI at first antenatal visit				
<20	312520	(11.0)	5139	(6.6)
20-24.9	1200271	(42.2)	27112	(34.9)
25-29.9	441373	(15.5)	16118	(20.8)
≥30	167717	(6.0)	10300	(13.3)
Missing	720349	(25.3)	18931	(24.4)
Optimal gestational weight gain by BMI group at first antenatal visit [(230)]				
<20				
Optimum	45641	(1.6)	514	(0.7)
Inadequate/Excessive	158243	(5.5)	2656	(3.4)
20-24.9				
Optimum	141430	(5.0)	1958	(2.5)
Inadequate	2563	(0.1)	43	(0.06)
Excessive	512433	(18.0)	12424	(16.0)
25-29.9				
Optimum	36368	(1.3)	803	(1.0)
Excessive	172818	(6.1)	6,996	(9.0)
≥30				
Optimum	14994	(0.5)	606	(0.8)
Excessive	58340	(2.1)	3893	(5.0)
Missing	1699400	(59.8)	47,707	(61.5)
Income quintile				
First	513347	(18.1)	11098	(14.3)
Second	532669	(18.7)	12625	(16.3)
Third	537973	(18.9)	14611	(18.8)
Fourth	540823	(19.0)	16657	(21.5)
Fifth	536843	(18.9)	17281	(22.3)
Missing	180575	(6.4)	5328	(6.8)
Parental level of education at IP birthyear (available from 1990)				
Pre-high school	132,995	(4.7)	3,346	(4.3)

High school	891,979	(31.4)	26,755	(34.5)
Post high school	888,712	(31.3)	24,098	(31.0)
Missing	928544	(32.7)	23401	(30.2)

Abbreviations: SGA=small for gestational age. BMI=body mass index. IP=index person.
Categories were collapsed if cell count <10, for example, inadequate/excessive weight gain in women categorised as BMI<20 were combined for the purpose of displaying data only.
If missing data >5%, number (%) of missing data reported.

Table 6.2 Association between preeclampsia and ASD with and without intellectual disability among singleton live births in Sweden between 1982 and 2010

	Total population			Sibling pairs
All ASD (n=54071)	**Exposed cases**	**Model 1 HR (95% CI)[a]**	**Model 2 HR (95% CI)[b]**	**Model 3 HR (95% CI)[c]**
Preeclampsia	2,024	1.36 (1.31, 1.43)	1.25 (1.19, 1.30)	1.17 (1.06, 1.28)
Preeclampsia and SGA[d]	326	1.79 (1.61, 2.00)	1.66 (1.49, 1.85)	1.95 (1.53, 2.48)
Preeclampsia without SGA	1673	1.32 (1.26, 1.38)	1.20 (1.14, 1.26)	1.11 (1.01, 1.23)
SGA only	1884	1.77 (1.69, 1.85)	1.60 (1.53, 1.67)	1.82 (1.65, 2.01)
ASD with intellectual disability (n=8981)				
Preeclampsia	388	1.59 (1.44, 1.76)	1.56 (1.41, 1.73)	1.32 (1.07, 1.62)
Preeclampsia and SGA[d]	90	3.11 (2.52, 3.82)	2.95 (2.40, 3.64)	3.07 (1.97, 4.79)
Preeclampsia without SGA	287	1.42 (1.26, 1.60)	1.40 (1.24, 1.57)	1.15 (0.91, 1.45)
ASD without intellectual disability (n=45090)				
Preeclampsia	1636	1.32 (1.26, 1.39)	1.19 (1.13, 1.25)	1.13 (1.01, 1.26)
Preeclampsia and SGA[d]	236	1.54 (1.36, 1.76)	1.42 (1.25, 1.62)	1.63 (1.22, 2.19)
Preeclampsia without SGA	1386	1.30 (1.23, 1.37)	1.17 (1.10, 1.23)	1.10 (0.98, 1.23)

Abbreviations: HR=hazard ratio. 95% CI=95% confidence interval. SGA=small for gestational age.
[a]Adjusted for year of birth.
[b]Adjusted for year of birth, infant sex, maternal age, maternal and paternal country of birth, birth order, parental depression, bipolar disorder and non-affective psychiatric disorders, maternal smoking status, BMI at first antenatal visit, gestational weight gain, family income and parental level of education.
[c]Adjusted for same potential confounders as above with the exception of maternal country of birth.
[d]Reference=no preeclampsia/no SGA.
Missing data on SGA for 25 cases of ASD (missing data on SGA for 11 cases of ASD with intellectual disability, and missing data on SGA for 14 cases of ASD without intellectual disability).

Table 6.3 Association between preeclampsia and ASD with and without intellectual disability among singleton live births in Sweden by decade

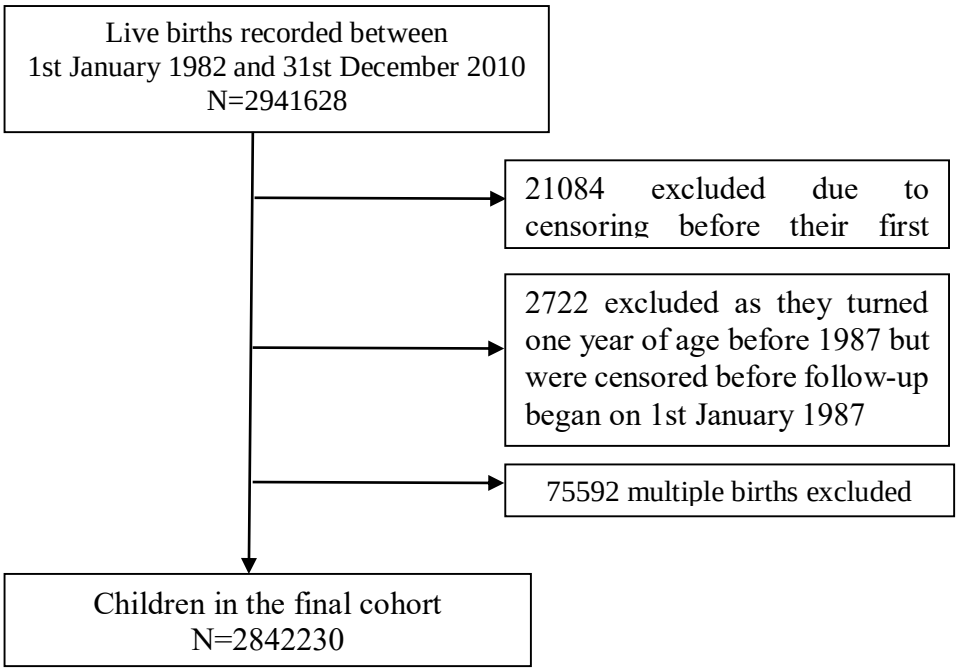
	Children born 1982-1989		**Children born 1990-1999**		**Children born 2000-2010**	
	ASD (n=10938)		**ASD** (n= 24237)		**ASD** (n= 18896)	
All ASD (n=54071)	**Exposed cases**	**Model 2 HR (95% CI)[a]**	**Exposed cases**	**Model 2 HR (95% CI)[a]**	**Exposed cases**	**Model 2 HR (95% CI)[a]**
Preeclampsia	336	1.15 (1.03, 1.28)	898	1.23 (1.15, 1.32)	790	1.30 (1.21, 1.39)
Preeclampsia and SGA[b]	50	1.34 (1.01, 1.77)	124	1.39 (1.16, 1.65)	152	2.14 (1.83, 2.51)
Preeclampsia without SGA	281	1.14 (1.01, 1.28)	760	1.21 (1.13, 1.31)	632	1.20 (1.11, 1.30)
ASD with intellectual disability (n=8981)						
Preeclampsia	60	1.38 (1.07, 1.80)	176	1.53 (1.32, 1.79)	152	1.64 (1.39, 1.94)
Preeclampsia and SGA[b]	14	2.57 (1.51, 4.36)	26	1.87 (1.27, 2.76)	50	4.39 (3.31, 5.81)
Preeclampsia without SGA	44	1.25 (0.92, 1.69)	144	1.52 (1.28, 1.79)	99	1.29 (1.06, 1.58)
ASD without intellectual disability (n=45090)						
Preeclampsia	276	1.11 (0.98, 1.25)	722	1.17 (1.09, 1.27)	638	1.23 (1.14, 1.34)
Preeclampsia and SGA[b]	36	1.13 (0.81, 1.56)	98	1.30 (1.06, 1.58)	102	1.71 (1.41, 2.08)
Preeclampsia without SGA	237	1.12 (0.98, 1.28)	616	1.16 (1.07, 1.26)	533	1.18 (1.08, 1.29)

Abbreviations: HR=hazard ratio. 95% CI=95% confidence interval. SGA=small for gestational age.

[a]Adjusted for year of birth, infant sex, maternal age, maternal and paternal country of birth, birth order, parental depression, bipolar disorder and non-affective psychiatric disorders, maternal smoking status, BMI at first antenatal visit, gestational weight gain, family income and parental level of education.

[b]Reference=no preeclampsia/no SGA

Figure 6-1 Flowchart of study participants



Chapter 7: ASSOCIATION BETWEEN PREECLAMPSIA AND ATTENTION DEFICIT HYPERACTIVITY DISORDER: A POPULATION-BASED AND SIBLING-MATCHED COHORT STUDY

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Published in: *Acta Psychiatrica Scandinavica (2020) (**Appendix 26**)*

7.1 Abstract

Objective: Examine the association between preeclampsia and attention deficit hyperactivity disorder (ADHD), using a large Swedish-based registry cohort.

Methods: This study comprised 2,047,619 children, with 114,934 (5.6%) cases of ADHD. Preeclampsia was based on two alternate definitions: 1. Preeclampsia (using ICD-9/ICD-10) 2. Preeclampsia and small for gestational age (SGA) combined. ADHD was determined in one of two ways: 1. If a diagnosis of ADHD was present in the National Patient Register or 2. If an individual was in receipt of ADHD medication in the Prescribed Drug Register. Multivariate Cox proportional hazards regression analysis allowed adjustment for several perinatal/sociodemographic factors. Sibling-matched analysis further controlled for shared genetic and familial confounding.

Results: In the adjusted Cox model, preeclampsia was associated with an increase in likelihood of ADHD (HR: 1.15, 95% CI: 1.12, 1.19). The HR for preeclampsia and those born SGA was 1.43 (95% CI: 1.31, 1.55) in the adjusted model, compared to those unexposed to preeclampsia/SGA. The sibling-matched analysis did not materially change these associations (HR: 1.13, 95% CI: 1.05, 1.22) and 1.55 (95% CI: 1.28, 1.88).

Conclusions: Exposure to preeclampsia or preeclampsia/SGA was associated with ADHD, independent of genetic/familial factors shared by siblings. However, it is important to note that sibling-matched analysis can only adjust for factors that are constant between pregnancies, therefore residual confounding cannot be ruled out. Further research is needed to explore modifiable risk factors and identify those most-at-risk babies following delivery.

7.2 Introduction

Preeclampsia, which affects approximately 5% of all pregnancies[4], is one of the leading causes of maternal morbidity and mortality, and was recently redefined by the International Society for the Study of Hypertension in Pregnancy (ISSHP) as gestational hypertension accompanied by at least two of the following: proteinuria and/or other maternal organ dysfunction and/or uteroplacental dysfunction[2].

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterised by inattention, hyperactivity and impulsivity. ADHD has a global pooled prevalence of over 5%, and while this estimate varies significantly worldwide, the variability can mostly be explained by methodological differences between studies[37, 38]. Despite high heritability estimates, gene environment interactions may also play a role[80].

Preeclampsia has been linked to adverse neurodevelopmental outcomes, including ADHD[43, 56]. Pooled results from a recent systematic review suggest that preeclampsia is associated with a 30% increase in odds of ADHD[56]. It is worth noting however, that while an apparent relationship exists in previous literature, residual confounding and quality of the studies may be a concern. For example, only one of ten studies included in the systematic review controlled for a combination of key potential confounders, such as maternal age, socioeconomic status, ethnicity, and maternal mental illness[40, 56].

Therefore, the aim of this study was to examine the association between preeclampsia and ADHD using a large population-based cohort study, controlling for a wide range of potential confounding factors, as well as shared genetic and familial confounding through sibling-matched analysis.

7.3 Methods

All singleton live births in Sweden from 1990 to 2010, with a follow-up until December 2016, were included in the study. Data were obtained from Swedish National Registers. These included the Medical Birth Register, National Patient Register, Prescribed Drug Register, Multi-generation Register, Total Population Register and Register of Education, linked using personal identification numbers assigned to Swedish residents[84].

Ethical approval was previously obtained from the Stockholm Regional Ethical Review Board (number 2010/1185-31/5), and informed consent was waived by the ethics committee.

Exposures

Preeclampsia

Data on preeclampsia was obtained from the Medical Birth Register which contains data on over 97% of all births in Sweden[85]. We used two alternate definitions of preeclampsia:

1. Preeclampsia: Blood pressure ≥140/90 mmHg on or after 20 weeks' gestation combined with proteinuria (≥0.3 g/day or ≥1 on a urine dipstick on at least two occasions). Preeclampsia was classified using the Swedish version of the ICD, Ninth and Tenth Revision[231]: ICD-9 until 1996 (codes 642E-G) and ICD-10 from 1997 (codes O14-O15)[16, 232].

2. Preeclampsia and small for gestational age (SGA) combined: We combined preeclampsia (as above) and SGA as a proxy for preeclampsia with placental dysfunction[2]. SGA was defined as birthweight <2 standard deviations below the mean of the sex-specific and gestational age distributions[98].

Outcome

Data on ADHD were obtained from the National Patient Register and the Prescribed Drug Register. The National Patient Register was launched in 1964, contains inpatient psychiatric diagnoses from 1973, and outpatient data since 2001 (with increasingly better coverage until 2006)[87, 88, 233]. The Prescribed Drug Register was expanded on 1st July 2005 to include personal identification numbers allowing linkage to other registers[88, 89].

A diagnosis of ADHD was determined in one of two ways:

1. If a diagnosis of ADHD was present in the National Patient Register, using ICD-10 (code F90 and F98.8), available since 1997[88].

2. If the subject was in receipt of ADHD medication in the Prescribed Drug Register. ADHD medication data was classified according to Anatomical Therapeutic Chemical classification system, and included amphetamine (N06BA01), dexamphetamine (N06BA02), psychostimulants methyphenidate (N06BA04) and noradrenergic reuptake inhibitor atomoxetine (N06BA09).

Confounding Factors

Potential confounders were based on previous literature. Year of birth, infant sex, maternal age, parental country of birth, parity, maternal smoking status, body mass index (BMI) at first antenatal visit and gestational weight gain were obtained from the Medical Birth Register. Parental depression, bipolar disorder, and non-affective psychiatric disorders were obtained from the National Patient Register. Family income and parental level of education data were obtained from the Total Population Register and Register of Education. Information on all cofounders was available for the entire study period. Where a variable had missing data, the data were added as a separate

category and included in the various Cox regression analyses by means of an indicator variable to ensure that all cases were included in the analyses[110]. (**Appendix 12**).

Statistical Analysis

All data were analysed using Stata/MP 14.2. We conducted Cox proportional hazards regression analysis to calculate a hazard ratio (HR) and 95% confidence interval for a preeclampsia-ADHD relationship, preeclampsia/SGA-ADHD (i.e SGA baby exposed to preeclampsia) relationship and the relationship between preeclampsia without SGA and ADHD.

Similar to a previous ADHD study conducted on this population (and because a diagnosis of ADHD is less likely to occur before this time)[88], follow-up began from a child's third birthday, (or 1st January 1997 for children who turned three years of age before 1997). Children continued to be followed up until he/she received a diagnosis of ADHD, prescription for ADHD, death, emigration, or the study period had ended (31st December 2016).

Partially adjusted models were stratified for year of birth in order to satisfy the proportional hazard assumption (model 1). Fully adjusted models (model 2) controlled for year of birth, infant sex, maternal age, parental country of birth, parity, parental depression, bipolar disorder, and non-affective psychiatric disorder, family income, maternal smoking status, BMI at first antenatal visit, gestational weight gain and parental level of education.

Sibling-matched analysis: We conducted a sibling-matched analysis (model 3) to control for shared genetic and familial confounding, using stratified Cox regression. This analysis was matched on maternal ID and consisted of a separate stratum for each family in order to estimate the probability of ADHD within family[51]. We adjusted for

the same potential confounders as model 2 with the exception of maternal country of birth as this is the same across sibling pairs. Finally, we repeated these analyses, firstly, including only those with both an ICD code for ADHD and if the subject was in receipt of ADHD medication, and secondly, including only those with an ICD code for ADHD.

Post-hoc analysis: We examined the association between SGA only and ADHD compared to non-exposure to SGA/non-exposure to preeclampsia.

E-value: We calculated the E-value for the statistically significant primary effect estimates and lower limits of their 95% confidence interval (CI) to examine the extent of unmeasured confounding, using the publicly available online E-value calculator: (https://evalue.hmdc.harvard.edu/app/)[234, 235]. In summary, an E-value is a continuous measure that quantifies the minimum strength of association an unmeasured confounder would need to have with both preeclampsia and ADHD in order to explain away an effect estimate[235].

Sensitivity analyses: We conducted several sensitivity analyses, decided *a priori*. For example, while classifying preeclampsia into mild/severe is not recommended in clinical practice because it is a complex disorder that can deteriorate rapidly, gestational age is sometimes used as a proxy for preeclampsia with severe features. As a result, preeclampsia could be considered severe if delivery occurred before 34 weeks' gestation[220]. Therefore, we examined the association between preeclampsia and ADHD by gestational age. In addition, it is possible that a mother's lifestyle factors could change between pregnancies. As a result, we excluded women who had preeclampsia in her first pregnancy, and examined a preeclampsia-ADHD relationship in women who had a diagnosis of preeclampsia in subsequent pregnancies only. Additional sensitivity analyses included restricting the study population to 2001-2010

(when outpatient data on ADHD started to become available), and restricting the study population to 1994-2010 to ensure every child begins follow-up at their third birthday. Furthermore, we included 'preeclampsia excluding chronic hypertension' as the exposure, and 'preeclampsia with chronic hypertension' as the exposure. We examined preeclampsia-ADHD excluding those with a family history of mental illness. We analysed the relationship between preeclampsia with low/intermediate APGAR score at five minutes, while we also examined a preeclampsia-ADHD relationship by maternal age, in addition to preeclampsia-ADHD by BMI group at time of first antenatal visit. Finally, we investigated a preeclampsia-ADHD association by gender.

7.4 Results

Descriptive Statistics

A total of 2,142,694 live births were recorded in the Swedish Medical Birth Register between 1990 and 2010. After excluding 61,172 multiple births, 30,636 children who were censored before their third birthday, and 3267 children who turned three years of age before 1997 but were censored before follow-up began on 1st January 1997, a total of 2,047,619 children remained in the final cohort (**Table 7.1**).

There were 57,493 (2.8%) children exposed to preeclampsia and 7191 (0.4%) exposed to preeclampsia and SGA combined. There were 114,934 (5.6%) cases of ADHD. Of these 101,075 (87.9%) cases were prescribed ADHD medication at some point, and 94,708 (82.4%) cases had an ICD diagnosis. A total of 80,849 (70.3%) cases were recorded with both an ICD code and medication, while there were 13,859 (12.1%) cases with an ICD code only, and 20,226 (17.6%) cases with medication only.

Association between preeclampsia, preeclampsia/SGA and ADHD

In the fully adjusted model (model 2), the results suggested an association between preeclampsia and ADHD (HR: 1.15, 95% CI: 1.12, 1.19) compared to those unexposed to preeclampsia. Result of the sibling-matched analysis (model 3) did not significantly change (HR: 1.13, 95% CI: 1.05, 1.22). The HR for those born SGA and exposed to preeclampsia was 1.43 (95% CI: 1.31, 1.55) in the adjusted model (model 2), and 1.55 (95% CI: 1.28, 1.88) in the sibling-match model (model 3), while the HR for those exposed to preeclampsia but not born SGA was 1.12 (95% CI: 1.08, 1.16) in model 2, and 1.09 (95% CI: 1.01, 1.18) in model 3. Limiting the data to those with both an ICD code and medication data did not materially change results, while including only those with an ICD code for ADHD produced similar results (**Table 7.2**).

Post-Hoc Analysis

The adjusted HR for SGA only (i.e. SGA without preeclampsia) and ADHD was 1.32 (95% CI: 1.27, 1.37), while the HR in the sibling-matched analysis was 1.29 (95% CI: 1.19, 1.39) compared to non-exposure to SGA/non-exposure to preeclampsia (**Table 7.2**).

E-Values

The E-values for significant primary effect estimates were 1.51 for preeclampsia, 2.47 for preeclampsia with SGA and 1.40 for preeclampsia without SGA, while the E-values for corresponding lower limits of their 95% CI were 1.28, 1.88 and 1.11 respectively. (see **Appendix 16** for worked example on preeclampsia-ADHD).

Sensitivity Analyses

Preeclampsia and ADHD by gestational age

When we restricted analysis to children born ≥39 weeks' gestational age, the HR for a preeclampsia-ADHD relationship was 1.07 (95% CI: 1.02, 1.12). Among children born at 37-38 weeks', the HR in those exposed to preeclampsia was 1.20 (95% CI: 1.13, 1.28), while the HR among those not exposed to preeclampsia was 1.09 (95% CI: 1.08, 1.11), when compared to non-exposure to preeclampsia in those born ≥39 weeks' gestational age. Exposure to preeclampsia (among children born 34-36 weeks') was associated with a 24% increase in likelihood of ADHD (HR: 1.24, 95% CI: 1.14, 1.35), while those unexposed to preeclampsia had a 14% increased likelihood of ADHD among those born at a similar gestational age (HR: 1.14, 95% CI: 1.11, 1.18). Finally, the HR among those exposed to preeclampsia (born <34 weeks' gestational age) was 1.74 (95% CI: 1.60, 1.91), while the HR among those not exposed to preeclampsia (born <34 weeks' gestational age) was 1.49 (95% CI: 1.42, 1.96) when compared to non-exposure to preeclampsia among those born ≥39 weeks' gestational age (**Table 7.3**).

Additional sensitivity analyses

Results of additional sensitivity analyses are outlined in **Appendix 17** and **Appendix 18** and were not materially different from the primary analysis. In sum, when we excluded women who had preeclampsia in their first pregnancy, the adjusted HR was 1.21. When we restricted the study population to 2001-2010 and 1994-2010, the HR was 1.21 and 1.14 respectively. The fully adjusted HR for preeclampsia (excluding chronic hypertension) and preeclampsia (with chronic hypertension) were 1.15 and 1.18 respectively. The HR for preeclampsia (excluding those with a family history of

mental illness was 1.16. Preeclampsia (with low/intermediate APGAR at 5 minutes) increased the likelihood of ADHD by 13% when compared to non-exposure to preeclampsia in those with a low/intermediate APGAR score. Results of the subgroup analysis suggested that preeclampsia was significantly associated with ADHD at each category of maternal age and at each category of BMI at first antenatal visit. The HR for preeclampsia-ADHD in males was 1.18 compared to non-exposure to preeclampsia in males, while the HR for preeclampsia-ADHD in females was 1.10 compared to non-exposure to preeclampsia in females. Finally, exposure to preeclampsia in males was associated with a 9% increase in likelihood of ADHD when compared to exposure to preeclampsia in females.

7.5 Discussion

The aim of this study was to examine the association between preeclampsia and preeclampsia/SGA and ADHD, using a large population-based cohort study. We have yielded three principal findings. First, after controlling for known potential confounding factors, preeclampsia was associated with a 15% increase in likelihood of ADHD when compared to non-exposure to preeclampsia. This finding was similar in the sibling-matched analysis suggesting that this apparent preeclampsia-ADHD relationship was not due to shared genetics or familial environment. This result is in line with the pooled estimate from a systematic review, which suggested that preeclampsia was associated with a 30% increase in odds of ADHD, with individual study estimates ranging from 1.19 to 1.50[(56)].

Second, as SGA is associated with uteroplacental dysfunction[(218)], and due to recent guidelines put forward by ISSHP to include uteroplacental dysfunction in the definition of preeclampsia, we combined preeclampsia and SGA into a single exposure

as a crude proxy for preeclampsia with placental dysfunction. Being an SGA baby and exposed to preeclampsia was associated with a 43% increase in likelihood of ADHD in the fully adjusted model, and a 55% increase in likelihood of ADHD in the sibling-matched analysis, when compared to non-exposure to preeclampsia or SGA. This may suggest that placental pathology may be a common factor increasing the likelihood of ADHD given the stronger association with preeclampsia/SGA than preeclampsia alone.

Three, preeclampsia was associated with ADHD, independent of gestational age. For example, preeclampsia was associated with a 7% increase in likelihood of ADHD when we restricted the analysis to those born ≥39 weeks' gestation. However, the HR increases to 1.74 among those exposed to preeclampsia and born at <34 weeks' gestation.

This apparent preeclampsia-ADHD association may lack specificity however, as preeclampsia is associated with several neurodevelopmental outcomes such as autism spectrum disorder (ASD), cognitive impairment and intellectual disability (ID) in previous literature[56]. Therefore, preeclampsia could in fact be a risk factor for poor neurodevelopmental outcome in general, with the specificity of outcome (e.g. ADHD, ASD, ID etc.) being determined by underlying genetic risk factors[200].

Potential Mechanisms

The molecular basis of a preeclampsia-ADHD relationship remains unknown, and there are few studies that address the potential biological mechanisms of ADHD specifically. Animal models have shown that activation of interleukin-17a (IL-17a) in the fetal brain, in response to maternal immune activation, is associated with behavioural disturbances and an abnormal cortical phenotype in affected offspring[22,

[236]. Therefore, we can speculate that maternal inflammation may be one such mechanism given the role of preeclampsia in chronic immune activation and elevated levels of inflammatory cytokines such as IL-17a[22, 73, 224]. In a separate study, maternal depressive symptoms throughout pregnancy were shown to be associated with ADHD in offspring[237]. As prenatal depression is linked to an increase in levels of pro-inflammatory cytokines[238], it is possible that the inflammatory response observed in preeclampsia may have a similar inflammatory mediated effect on ADHD-risk.

However, it may also be possible that lifestyle factors not available in the registers, such as maternal alcohol consumption may also play a role. Alcohol consumption during pregnancy has been shown to affect placentation, fetal growth, and likelihood of ADHD[239, 240]. As preeclampsia is, at least in part, a disease of placentation, leaving the fetus vulnerable to the effects of placental pathology, particularly fetal growth restriction[2], it is plausible that maternal alcohol consumption during pregnancy may contribute the observed preeclampsia-ADHD association.

Strengths and Limitations

There are several strengths in this study. To our knowledge, it is the largest epidemiological study to examine the association between preeclampsia-ADHD to date. Use of National Registers minimised recall bias, while also allowed us to control for a wide range of confounding factors. In addition, the sibling-matched analysis allowed us to adjust for unmeasured confounding factors shared by siblings such as family environment, diet, lifestyle factors, maternal characteristics, and genetic factors[98]. Furthermore, use of the E-value allowed us to quantify Bradford-Hill's consideration of 'strength of association' in an attempt to investigate the robustness of our effect estimates to unmeasured confounding[235].

However, this study also contains several limitations. First, sibling-matched analysis may have reduced confounding due to shared genetic and familial factors. However, this method can only adjust for factors that are constant between pregnancies[229] and the possibility of residual confounding cannot be ruled out in observational studies. Taking preeclampsia-ADHD as an example: (E-value for effect estimate = 1.51), an unmeasured confounder associated with both preeclampsia and ADHD by a risk-ratio of 1.51 may potentially explain away our preeclampsia-ADHD effect estimate of 1.13. However, the effect-estimate for preeclampsia/SGA combined is less likely to be explained away by unmeasured confounding with an E-value of 2.47. Nonetheless, we cannot dismiss the potential effect of factors such as maternal alcohol consumption could have on findings. Second, a lack of robust data on gestational hypertension limited our analysis to preeclampsia-ADHD only. Therefore, our comparison groups may contain women with a diagnosis of gestational hypertension, and while previous literature suggests a positive gestational hypertension-ADHD association[241], this would likely bias our results towards to the null. Third, as outpatient data only started becoming available in 2001, more severe cases of ADHD may have been overrepresented in our data. However, when we restricted the study population to 2001-2010, results were not materially different from our main findings suggesting that the inclusion of less severe cases after 2001 may not have had a large impact on findings.

Conclusion

This population-based cohort suggests that preeclampsia as well as preeclampsia/SGA was associated with ADHD. Placental pathology may be a common mechanism increasing the likelihood of ADHD given the stronger association with

preeclampsia/SGA, rather than preeclampsia alone. Further research is needed in order to clarify this association, explore modifiable risk factors and identify those most-at-risk babies following delivery.

Table 7.1 Perinatal and sociodemographic characteristics related to preeclampsia and ADHD among singleton live births in Sweden between 1990 and 2010

	No. (%) of Infants	
Characteristic	**Total Population**	**Preeclampsia**
Total Population	2047619	57493 (2.8)
ADHD	114934 (5.6)	3941 (6.9)
SGA	46719 (2.3)	7191 (12.6)
First-born child	879954 (42.9)	37642 (65.5)
Sex (male)	1052095 (51.4)	29938 (52.1)
Maternal age, years		
<20	41285 (2.0)	1535 (2.7)
20-29	1015666 (49.6)	29354 (51.1)
30-39	935055 (45.7)	24569 (42.7)
≥40	55613 (2.7)	2035 (3.5)
Gestational age, weeks		
<34	23538 (1.1)	5048 (8.8)
34	12181 (0.6)	1702 (3.0)
35	20845 (1.0)	2337 (4.1)
36	41472 (2.0)	3868 (6.7)
37	98759 (4.8)	6385 (11.2)
38	277445 (13.6)	9153 (15.9)
39	472125 (23.1)	10632 (18.5)
40	580209 (28.4)	10128 (17.6)
>40	519037 (25.4)	8162 (14.2)
5-Minute Apgar score		
0-3 (low)	3419 (0.2)	228 (0.4)
4-6 (intermediate)	15330 (0.8)	1251 (2.2)
7-10 (high)	2013115 (99.0)	55464 (97.4)
Mother's country of birth		
Sweden	1597528 (78.0)	47286 (82.2)
Other Nordic country	44704 (2.2)	1301 (2.3)
Other country	278978 (13.6)	5709 (9.9)
Missing	126409 (6.2)	3197 (5.6)
Father's country of birth		
Sweden	1577672 (77.1)	46891 (81.6)
Other Nordic country	42429 (2.1)	1184 (2.0)
Other country	287522 (14.0)	5820 (10.1)
Missing	139996 (6.8)	3598 (6.3)
Maternal depression		
Never	1763485 (86.1)	49730 (86.5)
Previously diagnosed	157876 (7.7)	4574 (7.9)
Missing	126258 (6.2)	3189 (5.6)
Maternal bipolar disorder		
Never	1904427 (93.0)	53772 (93.5)
Previously diagnosed	16934 (0.8)	532 (0.9)
Missing	126258 (6.2)	3189 (5.6)
Maternal nonaffective disorder		
Never	1909156 (93.2)	53923 (93.8)
Previously diagnosed	12205 (0.6)	381 (0.6)
Missing	126258 (6.2)	3189 (5.6)
Paternal depression		
Never	1831285 (89.4)	51886 (90.2)
Previously diagnosed	90076 (4.4)	2418 (4.2)
Missing	126258 (6.2)	3189 (5.6)
Paternal bipolar disorder		

Never	1911454 (93.3)	54058 (94.0)
Previously diagnosed	9907 (0.5)	246 (0.4)
Missing	126258 (6.2)	3189 (5.6)
Paternal nonaffective disorder		
Never	1909156 (93.2)	53980 (93.9)
Previously diagnosed	12205 (0.6)	324 (0.5)
Missing	126258 (6.2)	3189 (5.6)
Income quintile		
First	362540 (17.7)	8168 (14.2)
Second	383691 (18.7)	9542 (16.6)
Third	388138 (19.0)	11044 (19.2)
Fourth	390219 (19.1)	12509 (21.8)
Fifth	384890 (18.8)	12772 (22.2)
Missing	138141 (6.7)	3458 (6.0)
Smoking at first antenatal visit		
No	1683882 (86.4)	49417 (90.7)
1-9 cigarettes/day	178176 (9.1)	3576 (6.5)
≥10 cigarettes/day	87699 (4.5)	1515 (2.8)
BMI at first antenatal visit		
<20	172519 (8.4)	3048 (5.3)
20-24.9	868599 (42.4)	19449 (33.8)
25-29.9	372026 (18.2)	13037 (22.7)
≥30	154136 (7.5)	9415 (16.4)
Missing	480339 (23.5)	12544 (21.8)
Optimal gestational weight gain by BMI group at first antenatal visit[230]		
<20		
Optimum	15910 (0.8)	211 (0.4)
Inadequate/Excessive	49130 (2.4)	891 (1.6)
20-24.9		
Optimum	75448 (3.7)	1003 (1.7)
Inadequate/Excessive	254217 (12.4)	5855 (10.2)
25-29.9		
Optimum	25752 (1.3)	527 (0.9)
Excessive	115893 (5.7)	4260 (7.4)
≥30		
Optimum	12147 (0.6)	461 (0.8)
Excessive	48240 (2.3)	3180 (5.5)
Missing	1450882 (70.8)	41105 (71.5)
Highest parental level of education at child's birthyear		
Pre-high school	131210 (6.4)	3304 (5.7)
High school	886656 (43.3)	26603 (46.3)
Post high school	877980 (42.9)	23844 (41.5)
Missing	151773 (7.4)	3742 (6.5)

Abbreviations: SGA=small for gestational age. BMI=body mass index.
Categories were collapsed if cell count <10, for example, inadequate/excessive weight gain in women categorised as BMI<20 were combined for the purpose of displaying data only.
If missing data >5%, number (%) of missing data reported.

Table 7.2 Association between preeclampsia and ADHD among singleton live births in Sweden between 1990 and 2010

	Total population			Sibling pairs
All ADHD (n=114934)	**Exposed cases**	**Model 1 HR (95% CI)**[a]	**Model 2 HR (95% CI)**[b]	**Model 3 HR (95% CI)**[c]
Preeclampsia	3941	1.22 (1.18, 1.26)	1.15 (1.12, 1.19)	1.13 (1.05, 1.22)
Preeclampsia and SGA[d]	582	1.49 (1.37, 1.61)	1.43 (1.31, 1.55)	1.55 (1.28, 1.88)
Preeclampsia without SGA	3322	1.19 (1.15, 1.23)	1.12 (1.08, 1.16)	1.09 (1.01, 1.18)
SGA without Preeclampsia	3205	1.51 (1.45, 1.56)	1.32 (1.27, 1.37)	1.29 (1.19, 1.39)
ADHD (ICD code and in receipt of medication) (n=80849)				
Preeclampsia	2795	1.23 (1.18, 1.27)	1.16 (1.11, 1.20)	1.11 (1.02, 1.21)
Preeclampsia and SGA[d]	399	1.44 (1.31, 1.59)	1.37 (1.25, 1.52)	1.54 (1.23, 1.92)
Preeclampsia without SGA	2370	1.21 (1.16, 1.26)	1.13 (1.08, 1.18)	1.07 (0.98, 1.17)
ADHD (ICD code only) (n=94708)				
Preeclampsia	3267	1.23 (1.18, 1.27)	1.16 (1.12, 1.20)	1.11 (1.03, 1.20)
Preeclampsia and SGA[d]	480	1.48 (1.35, 1.62)	1.41 (1.29, 1.55)	1.48 (1.21, 1.82)
Preeclampsia without SGA	2757	1.20 (1.16, 1.25)	1.13 (1.09, 1.18)	1.07 (0.99, 1.17)

Abbreviations: HR=hazard ratio. 95% CI=95% confidence interval. SGA=small for gestational age. ICD=International Classification of Disease.

[a]Adjusted for year of birth.

[b]Adjusted for year of birth, infant sex, maternal age, parental country of birth, parity, parental depression, bipolar disorder, and non-affective psychiatric disorders, family income, maternal smoking status, BMI at first antenatal visit, gestational weight gain and parental level of education.

[c]Adjusted for same potential confounders as above with the exception of maternal country of birth.

[d]Reference=no preeclampsia/no SGA.

Missing data on SGA for 37 cases of ADHD (full cohort). Missing data on SGA for 26 cases of ADHD (with both ICD code and medication data). Missing data on SGA for 30 cases of ADHD (with ICD code).

Table 7.3 Association between preeclampsia and ADHD among singleton live births in Sweden between 1990 and 2010 by gestational age

	Total population		
All ADHD (n=114934)	**Exposed cases**	**Model 1 HR (95% CI)**[a]	**Model 2 HR (95% CI)**[b]
No Preeclampsia, ≥39 weeks' gestational age (ref)	82844	1.00	1.00
Preeclampsia, ≥39 weeks' gestational age	1808	1.14 (1.09, 1.20)	1.07 (1.02, 1.12)
No Preeclampsia, 37-38 weeks' gestational age	21742	1.13 (1.12, 1.15)	1.09 (1.08, 1.11)
Preeclampsia, 37-38 weeks' gestational age	1066	1.28 (1.20, 1.36)	1.20 (1.13, 1.28)
No Preeclampsia, 34-36 weeks' gestational age	4545	1.28 (1.24, 1.32)	1.14 (1.11, 1.18)
Preeclampsia, 34-36 weeks' gestational age	568	1.32 (1.22, 1.44)	1.24 (1.14, 1.35)
No Preeclampsia, <34 weeks' gestational age	1703	1.78 (1.70, 1.87)	1.49 (1.42, 1.56)
Preeclampsia, <34 weeks' gestational age	491	1.85 (1.69, 2.02)	1.74 (1.60, 1.91)

Abbreviations: HR=hazard ratio. 95% CI=95% confidence interval. ref=reference category. SGA=small for gestational age.

[a]Adjusted for year of birth.

[b]Adjusted for year of birth, infant sex, maternal age, parental country of birth, parity, parental depression, bipolar disorder, and non-affective psychiatric disorders, family income, maternal smoking status, BMI at first antenatal visit, gestational weight gain and parental level of education.

Missing data on gestational age for 167 cases of ADHD.

Chapter 10: UPDATED SYSTEMATIC REVIEW AND META-ANALYSIS FOR ASD AND ADHD

10.1 Introduction

An updated search of the literature was conducted using similar methods as outlined in Chapter 4. As discussed in the original systematic review, it is possible that keywords such as "perinatal complication" OR "prenatal complication" OR "obstetric* complication" were lacking in the original search. Therefore, we included these words in the updated search. A search PubMed, CINAHL, EMBASE, PsycINFO and Web of Science was performed from June 7, 2017 through 17th January 2020.

After removal of duplicates, this resulted in 837 new titles and abstracts to screen for inclusion in the updated systematic review. Of these, four new ASD studies (including the study presented in Chapter 6), and three new ADHD studies (including the study presented in Chapter 7) were identified.

Among the ASD studies, two case-control studies[267, 268] (one US multisite study and one study from Northern Taiwan), and two cohort studies[242, 269] (one from South California and our Swedish-based study presented in Chapter 6) were included. As one of the studies included in the original review examined a preeclampsia-ASD relationship based on data from the Swedish National Registers[14], this study was excluded in the updated analysis.

Among the ADHD studies, three cohort studies were included in the update[43, 241, 243] (one multi-centre European cohort, one study from United Kingdom, and our Swedish-based study presented in Chapter 7). Similar to above, as one of the studies included in the original review examined a preeclampsia-ADHD relationship based on data from the Swedish National Registers[44], this study was excluded in the updated analysis.

Both crude and adjusted estimates were included in the updated forest plots. As chapters 6 and 7 include traditional statistical adjustment and sibling-matched

analysis, the latter was considered the 'fully adjusted' result for inclusion in the updated analysis.

10.2 Updated meta-analysis results

10.2.1 Autism Spectrum Disorder

The updated, crude pooled estimate examining a HDP-ASD relationship was 1.43 (95% CI: 1.29, 1.58). The subgroup analyses, examining a preeclampsia-ASD and other HDP-ASD relationship separately, resulted in an OR of 1.43 (95% CI: 1.24, 1.64), and 1.47 (95% CI: 1.22, 1.76) respectively. These results were slightly higher than the crude estimates in the original systematic review, however not significantly different. (**Table 10.1** and **Figure 10.1**).

The updated, adjusted pooled estimate was 1.33 (95% CI: 1.17, 1.52). Subgroup analysis examining the preeclampsia-ASD relationship resulted in an OR of 1.36 (95% CI: 1.18, 1.58), while the relationship between other HDP-ASD produced a non-significant OR of 1.29 (95% CI: 0.97, 1.71). Again, these results were not materially different from those in the original systematic review. (**Table 10.1** and **Figure 10.1**).

10.2.2 Attention Deficit Hyperactivity Disorder

The crude pooled estimate suggested that HDP was associated with an over 30% increased odds of ADHD when compared to those unexposed (OR: 1.31, 95% CI: 1.22, 1.42). In the subgroup analyses examining preeclampsia-ADHD, and other HDP-ADHD, the OR was 1.29 (95% CI: 1.20, 1.40) and 1.76 (95% CI: 1.27, 2.46) respectively. Adjusted pooled estimates suggested HDP was associated with 26% increased odds of ADHD (OR: 1.26, 95% CI: 1.15, 1.38). For the subgroup analysis examining the preeclampsia-ADHD relationship, the OR was 1.23 (95% CI: 1.13,

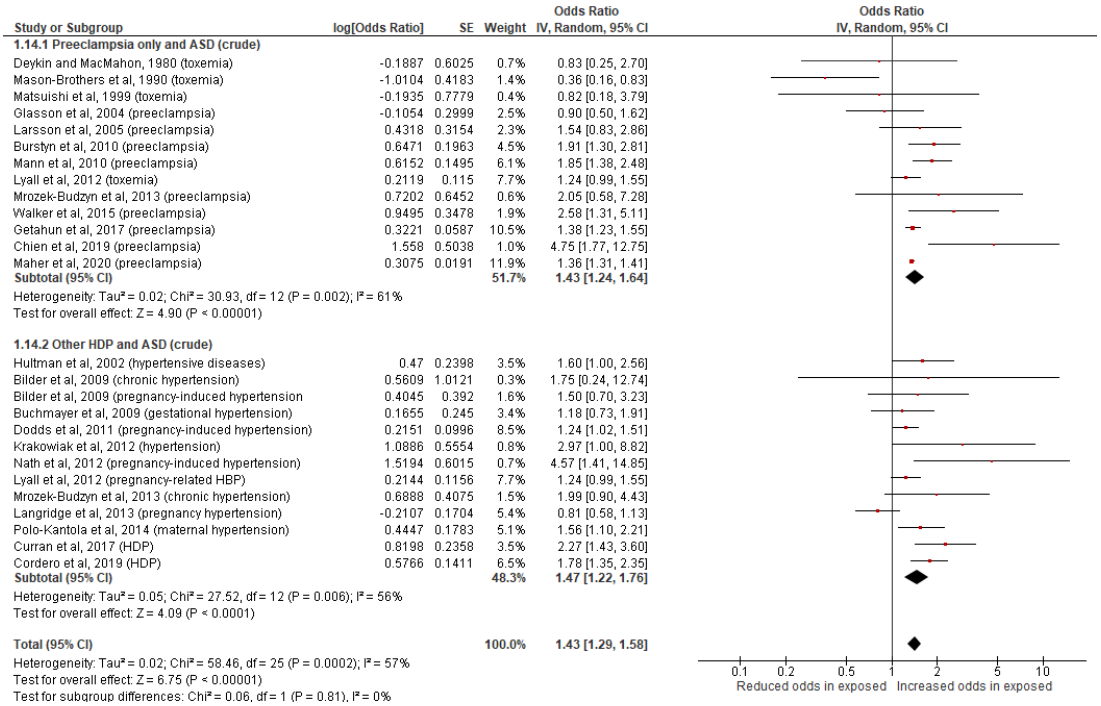
1.35), and for other HDP-ADHD relationship, the OR was 1.80 (95% CI: 1.25, 2.59). These results are similar to what was observed in the original systematic review. (**Table 10.1** and **Figure 10.2**).

Table 10.1 Comparison of pooled results between the original systematic review and updated systematic review

ASD	**Original systematic review**	**Updated systematic review**
Crude results	OR (95% CI)	OR (95% CI)
Overall HDP-ASD	1.41 (1.22, 1.64)	1.43 (1.29, 1.58)
Preeclampsia-ASD	1.37 (1.07, 1.75)	1.43 (1.24, 1.64)
Other HDP-ASD	1.43 (1.17, 1.73)	1.47 (1.22, 1.76)
Adjusted results		
Overall HDP-ASD	1.35 (1.11, 1.64)	1.33 (1.17, 1.52)
Preeclampsia-ASD	1.50 (1.26, 1.78)	1.36 (1.18, 1.58)
Other HDP-ASD	1.25 (0.90, 1.73)	1.29 (0.97, 1.71)
ADHD		
Crude results		
Overall HDP-ADHD	1.32 (1.20, 1.45)	1.31 (1.22, 1.42)
Preeclampsia-ADHD	1.31 (1.19, 1.44)	1.29 (1.20, 1.40)
Other HDP-ADHD	1.62 (1.07, 2.47)	1.76 (1.27, 2.46)
Adjusted results		
Overall HDP-ADHD	1.29 (1.22, 1.36)	1.26 (1.15, 1.38)
Preeclampsia-ADHD	1.28 (1.22, 1.36)	1.23 (1.13, 1.35)
Other HDP-ADHD	1.70 (1.06, 2.72)	1.80 (1.25, 2.59)
Abbreviations: OR=odds ratio. 95% CI=95% confidence interval.		

Figure 10-1 Updated forest plots of the association between HDP and ASD

Crude and partially adjusted estimates



Adjusted estimates

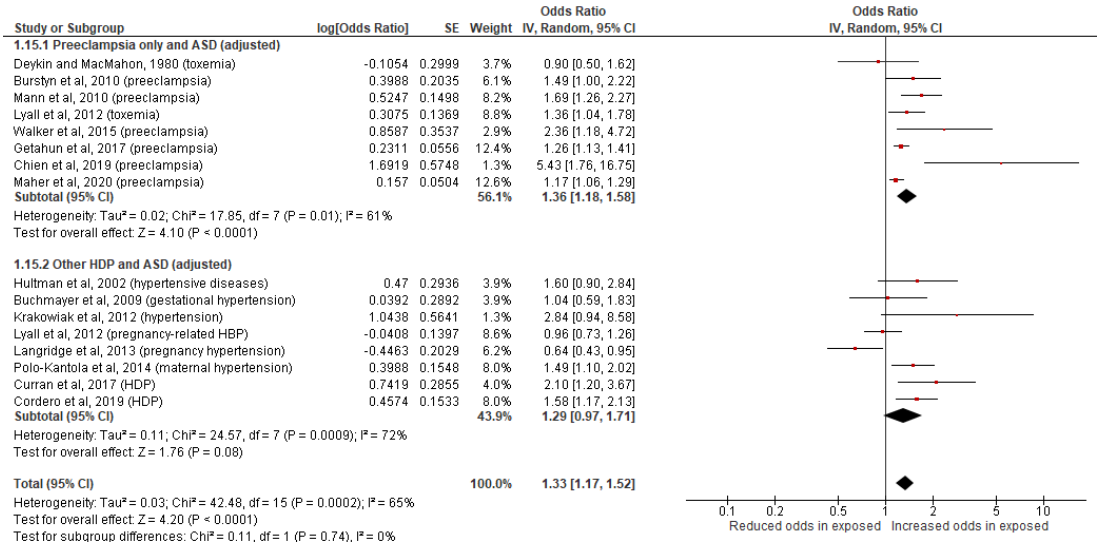
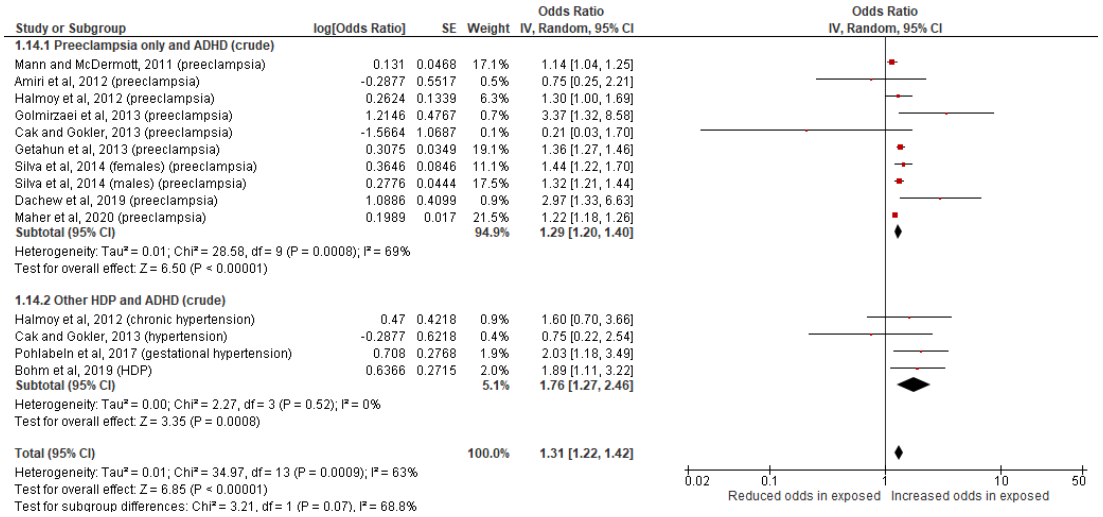
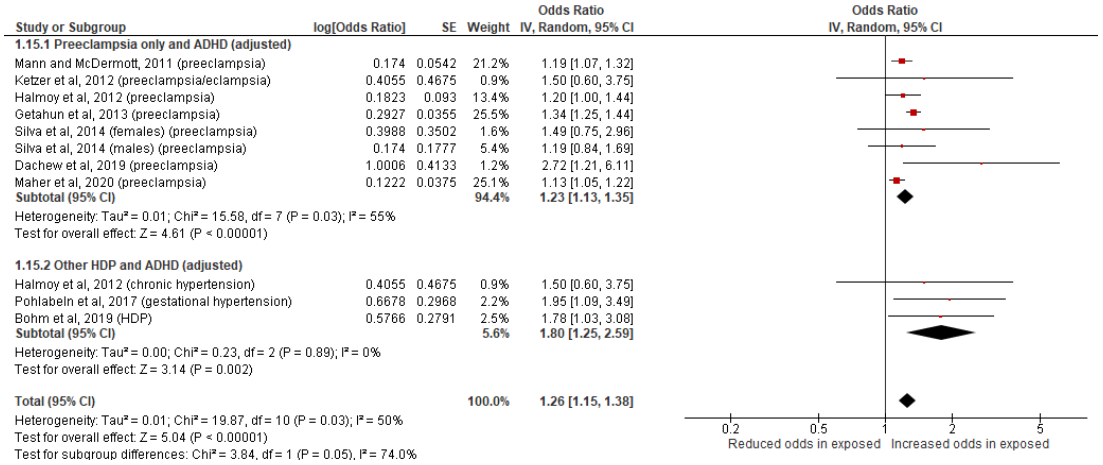


Figure 10-2 Updated forest plots of the association between HDP and ADHD

Crude and partially adjusted estimates



Adjusted estimates



Chapter 11: DISCUSSION

11.1 Summary of Main Findings

This thesis aimed to examine the association between HDP, in particular preeclampsia, and neurodevelopmental disorders in offspring. Specifically, this thesis includes a systematic review and meta-analysis of available published literature (based on a pre-prepared protocol), and a narrative review providing a perspective on how maternal inflammation may lead to altered neurodevelopmental outcomes in preeclampsia-exposed offspring. Also contained within this thesis are original investigations examining the association between preeclampsia and ASD, and ADHD (using Swedish National Registers), and child development and behavioural outcomes (using data from the Growing Up in Ireland study). This chapter reviews the findings of our analyses, and discusses the strengths and limitations, future directions and overall conclusions.

11.1.1 Findings from Systematic Review and Meta-analysis

A systematic review and meta-analysis was conducted to synthesise the available published literature on the relationship between HDP and neurodevelopmental disorders in the offspring (chapter 4). The systematic review was based on a pre-prepared protocol (chapter 3) and included studies until June 2017.

A total of 20 ASD-studies were identified for inclusion in the systematic review. Of these, 11 unique studies reported adjusted estimates. Pooled adjusted estimates suggest that exposure to HDP is associated with a 35% increased odds of ASD when compared to those unexposed (OR of 1.35, 95% CI: 1.11, 1.64). A subgroup analysis of adjusted results examining a preeclampsia-ASD relationship resulted in an OR of 1.50 (95% CI: 1.26, 1.78), while the relationship between other HDP-ASD was non-significant with an OR of 1.25 (95% CI: 0.90, 1.73). The updated search of the literature identified

four new ASD studies (including the study presented in Chapter 6). However, pooled results were not materially different from results observed in the original review (i.e. adjusted pooled estimate was 1.33 (95% CI: 1.17, 1.52), subgroup analyses examining preeclampsia-ASD and other HDP-ASD resulted in ORs of 1.36 (95% CI: 1.18, 1.58), and 1.29 (95% CI: 0.97, 1.71) respectively. (**Table 10.1** and **Figure 10.1**).

For ADHD, ten studies were identified in which a diagnosis of HDP was reported and ADHD was the outcome of interest. Of these, six unique studies included adjusted estimates. Adjusted pooled estimates suggested that offspring exposed to HDP are almost 30% more likely to be diagnosed with ADHD compared to those unexposed (OR: 1.29, 95% CI: 1.22, 1.36). Adjusted results of the subgroup analysis examining a preeclampsia-ADHD relationship and other HDP-ADHD relationship produced an OR of 1.28 (95% CI: 1.22, 1.36), and 1.70 (95% CI: 1.06, 2.72) respectively. Three new studies on ADHD were identified in the updated search (including the study presented in Chapter 7) and included in the analysis. The updated adjusted results indicated that exposure to HDP was associated with a 26% increased odds of ADHD (OR: 1.26, 95% CI: 1.15, 1.38). The subgroup analysis examining preeclampsia-ADHD and other HDP-ADHD resulted in an OR of 1.23 (95% CI: 1.13, 1.35) and 1.80 (95% CI: 1.25, 2.59) respectively. Therefore, updated pooled results were not significantly different from the original systematic review. (**Table 10.1** and **Figure 10.2**).

Results from previous literature examining the relationship between HDP and other neurodevelopmental, cognitive or behavioural outcomes could not be pooled due to methodological differences between studies, particularly differences in population and outcome assessment methods. Results were inconsistent, however some patterns of

association were observed between preeclampsia and cognitive impairment[57, 58, 60, 61, 69, 149], as well as a potential link between HDP and intellectual disability[36, 62, 63, 150]. The systematic review also highlighted some important limitations of previous literature, which we attempted to address in this thesis. For example, previous literature was often limited by small sample sizes as a quarter of ASD-studies and almost a third of ADHD-studies had fewer than ten exposed cases. Furthermore, residual or unmeasured confounding is of particular concern. There were some studies that failed to control for any potential confounding factors in the analysis phase of the study, and only one ASD-study[21] and one ADHD-study[40] controlled for a combination of potential confounders such as maternal age, socio-economic status, ethnic origin and family history of mental illness. Therefore, while results of the systematic review and meta-analysis indicate that exposure to HDP is associated with a small increase in the likelihood of ASD and ADHD, more robust research was needed addressing key limitations in the literature before more definitive conclusions could be reached.

11.1.2 Findings on Autism Spectrum Disorder

In chapter 6, we examined the association between preeclampsia and ASD using data from Swedish National Registers, and yielded two principal findings. First, adjusted results suggest that exposure to preeclampsia was associated with 25% increased odds of ASD when compared to those unexposed, after controlling for potential confounding factors (HR: 1.25, 95% CI: 1.19, 1.30). The sibling-matched analysis allowed us to further control for shared genetic and familial factors and reduced the HR to 1.17 (95% CI: 1.06, 1.28). However, when results were stratified by ASD with

and without intellectual disability, the HRs were 1.32 (95% CI: 1.07, 1.62) and 1.13 (95% CI: 1.01, 1.26) respectively, in the sibling-matched analysis.

Second, results of the sibling-matched analysis suggest that being an SGA baby and exposed to preeclampsia was associated with a 95% increased odds of ASD when compared to non-exposure to preeclampsia or SGA (95% CI: 1.53, 2.48). This HR increased to 3.07, when stratified by ASD with intellectual disability, and reduced to 1.63 when stratified by ASD without intellectual disability.

Preeclampsia is, at least in part, a disease of placentation and/or uteroplacental dysfunction. As a result, exposure to preeclampsia may leave the fetus vulnerable to the effects of placental pathology, particularly fetal growth restriction[2, 218]. As the definition of SGA in the Swedish Medical Birth Register (birthweight <2 standard deviations below the mean of the sex-specific and gestational age distributions)[98] approximately represents infants born with a birthweight <2.5th percentile, this could also be an indicator of intrauterine growth restriction (IUGR)[270]. Therefore, the observed preeclampsia and SGA relationship with ASD suggests that placental pathology may be a mechanism for the increased likelihood of ASD. On further investigation, our post-hoc analysis examining SGA-alone and ASD (HR: 1.82, 95% CI: 1.65, 2.01) supports this hypothesised mechanism given the modest effect of preeclampsia on likelihood of ASD compared to that of preeclampsia and SGA combined, or SGA-alone.

Furthermore, as recent guidelines proposed by ISSHP now include uteroplacental dysfunction (such as fetal growth restriction) in the definition of preeclampsia[2], it could be argued that a diagnosis of preeclampsia and SGA combined could be characterised as a more "severe" phenotype of preeclampsia, leading to an increased likelihood of ASD when compared to preeclampsia alone. In addition to this, results

of a sensitivity analysis (**Appendix 14**) suggested that exposure to preeclampsia in those born at <34 weeks' gestational age (used as a proxy for preeclampsia with severe features)[220] were over twice as likely to be diagnosed with ASD when compared to non-exposure to preeclampsia in those born at ≥34 weeks' gestation (HR: 2.04, 95% CI: 1.81, 2.30). This analysis further supports the notion that preeclampsia with severe features may lead to a stronger association with ASD.

With regards to the stronger association when results were stratified by ASD with intellectual disability, this could potentially be explained by increased circulating levels of maternal cytokines and chemokines in pregnancies complicated by preeclampsia[222]. Previous evidence suggests that mothers of children with ASD with intellectual disability had significantly elevated mid-gestational levels of numerous cytokines and chemokines, such as GM-CSF, IFN-γ, IL-1α, and IL-6, compared to mothers of children with ASD without intellectual disability, developmental delay, or general population controls[222]. These results are indicative of maternal immune activation leading to a shift in the immune balance during pregnancy, potentially affecting developmental programming and the neurodevelopmental trajectory of the child[222].

11.1.3 Findings on Attention Deficit Hyperactivity Disorder

In chapter 7, we examined the association between preeclampsia and ADHD using data from Swedish National Registers. After controlling for several perinatal and sociodemographic factors, preeclampsia was associated with a 15% increase in likelihood of ADHD when compared to non-exposure to preeclampsia (95% CI: 1.12, 1.19). Results of the sibling-matched analysis were not materially different from the main findings (HR: 1.13, 95% CI: 1.05, 1.22).

Being an SGA baby and exposed to preeclampsia further increased the likelihood of ADHD (HR: 1.55, 95% CI: 1.28, 1.88), however to a lesser extent to that of the ASD study. Moreover, results suggest that the likelihood of ADHD increases with decreasing gestational age, as preeclampsia was associated with a 7% increase in likelihood of ADHD when we restricted the analysis to those born ≥39 weeks' gestation (95% CI: 1.02, 1.12). However, the HR increases to 1.74 among those exposed to preeclampsia and born at <34 weeks' gestation (95% CI: 1.60, 1.91). Similar to the ASD study (above), these results suggest that preeclampsia with severe features may lead to stronger associations with neurodevelopmental outcomes.

There are few studies that address the potential biological mechanisms of ADHD specifically. However, animal models have speculated that maternal inflammation may be one such mechanism given the role of preeclampsia in chronic immune activation and elevated levels of inflammatory cytokines such as IL-17a[22, 73, 224] which is associated with behavioural disturbances and an abnormal cortical phenotype in affected offspring[22, 236].

11.1.4 Findings on Intergenerational Association (ASD and ADHD)

As previous research indicates that risk of certain outcomes can be transferred across generations[52, 53], chapter 8 of this thesis examined the intergenerational association between preeclampsia and ASD and ADHD using data from Swedish National Registers. Similar to our previous findings on ASD and ADHD, exposure to preeclampsia was associated with an increased likelihood of ASD (HR: 1.31, 95% CI: 1.19, 1.43) and ADHD (HR: 1.23, 95% CI: 1.16, 1.30) in offspring. In addition to this, results suggested that preeclampsia in both the child's mother and grandmother

increased the likelihood of ASD (HR: 1.59, 95% CI: 1.03, 2.47) and ADHD (HR: 1.34, 95% CI: 1.01, 1.80) in the child.

These results suggest that preeclampsia may be associated with adverse neurodevelopmental outcomes across generations given the stronger association when a diagnosis of preeclampsia was present in both the child's mother and grandmother. However, results may also be indicative of a dose-response relationship. Women diagnosed as having preeclampsia with severe features are more likely to have been born of a pregnancy complicated by preeclampsia[244]. Therefore, it is plausible that a more "severe" phenotype of preeclampsia could be leading to the stronger intergenerational association in our study. This is in line with our previous suggested hypothesis that a more "severe" phenotype of preeclampsia may lead to an increase in the likelihood of adverse neurodevelopmental outcome.

11.1.5 Findings on Child Development and Behavioural Outcomes

Chapter 9 of this thesis examined the association between preeclampsia and child development (using the ASQ) at age 9-months, and preeclampsia and emotional/behavioural problems (using the SDQ) at age 3 years, 5 years and 7-8 years using data from Growing Up in Ireland (GUI), a nationally representative longitudinal study of children living in Ireland.

Adjusted results suggest preeclampsia was not associated with failing an ASQ domain at 9-months old. In addition to this, preeclampsia was not significantly associated with abnormal SDQ score in any of the domains at age 3 years and age 7-8 years. However, at age 5 years, preeclampsia was associated with abnormal SDQ cut-off of Emotional (OR: 1.50, 95% CI: 1.04, 2.17) and Hyperactivity (1.57, 95% CI: 1.19, 2.08) domains. These results support the notion that young children can sometimes transition in or out

of the abnormal range for behavioural issues throughout childhood[256]. Furthermore, results are consistent with brain imaging studies conducted on young children. For example, previous evidence suggests an association between exposure to preeclampsia and altered anatomical and functional connectivity in the amygdala and other regions of the 'social brain'[81, 206, 246]. Therefore, this may partly explain the association between preeclampsia and failure of the Emotional domain of the SDQ in particular.

11.2 Strengths and Limitations

11.2.1 Strengths of Thesis (overall)

This thesis includes a comprehensive systematic review and meta-analysis of the epidemiological evidence examining the association between HDP and neurodevelopmental outcomes. The systematic review and meta-analysis was updated upon completion of this thesis to include the current results and any newly published studies for ASD and ADHD (**Figure 10.1** and **Figure 10.2**). Also included in this thesis is a narrative review of the potential role of maternal inflammation in the development of ASD.

In addition, this thesis addresses the limitations of previous literature identified in the systematic review, using the largest epidemiological studies, to date, to examine a preeclampsia-ASD and preeclampsia-ADHD relationship. This work was complemented by using data from the GUI study to examine the associations between preeclampsia and child development and behavioural outcomes, as information on these outcomes is not available in the Swedish National Registers.

Furthermore, recent ISSHP guidelines suggest that uteroplacental dysfunction should be included in the definition of preeclampsia[2]. Combining preeclampsia and SGA as a crude proxy for preeclampsia with placental dysfunction is in line with these

guidelines given that the most frequent etiology for fetal growth restriction is uteroplacental dysfunction[271]. Finally, this thesis includes the first studies to conduct sibling-matched analyses when examining a preeclampsia-ASD and preeclampsia-ADHD relationship.

11.2.2 Strengths of Swedish National Register Studies

The Swedish data studies in this thesis were the largest epidemiological studies to date to investigate a preeclampsia-ASD/ADHD association. For ASD, all singleton live births in Sweden from 1982-2010 were included, while for ADHD, all singleton live births from 1990 to 2010 were included, with follow-up until 2016 for both. To our knowledge, this is the longest study period that has been covered on this topic.

As data were prospectively obtained from national registers, it minimised the likelihood of recall bias and selection bias. The national registers contained information on several potential confounding factors, an issue that was identified in previous literature, allowing us to control for a wide range of confounding variables. Moreover, conducting a sibling-matched analysis, allowed us to further control, at least in part, for shared genetic and familial factors.

In addition, evidence suggests that risk factors for ASD with and without intellectual disability may differ. As the National Patient Register contained information on intellectual disability, it allowed us to examine the association between preeclampsia and ASD overall, and stratified by ASD with and without intellectual disability[36, 216]. Furthermore, while a diagnosis of ADHD was determined using either ICD-coding or being in receipt of ADHD medication, we conducted a sensitivity analysis including only those with both a diagnosis using ICD-coding and ADHD medication, thus reducing the likelihood of misclassification bias. Finally, the large sample size allowed

several *a priori* sensitivity analyses to be conducted in an attempt to explain the observed associations.

11.2.3 Strengths of Growing Up in Ireland Study

The GUI study in this thesis used data from a nationally representative study of children living in Ireland, reducing the likelihood of selection bias. All data were weighted to represent the national sample of infants aged less than one year in the 2008 calendar year. We conducted repeated measures analysis using linear spline multilevel modelling, allowing for change in SDQ score over time. To my knowledge, this has not previously been conducted in studies examining a preeclampsia-behavioural outcome relationship. Finally, the GUI study data contained information on a wide range of potential confounders allowing us to control for several important confounding factors.

11.2.4 Limitations of Thesis (overall)

The systematic review and meta-analysis included English-language studies only; therefore, it is possible that non-English studies on this topic were overlooked. Moreover, we did not have access to quality data on gestational hypertension in the Swedish National Registers or GUI study, which limited our analyses and meant that the comparison groups may have contained women with a diagnosis of gestational hypertension. However, we are confident that if an association between gestational hypertension and neurodevelopment outcome were to exist, this would likely bias our results towards the null. In addition to this, as the studies included in this thesis are based on existing data, the ability to control for confounding factors is limited to the data available in the various datasets.

Finally, as discussed in 'Future Directions' below, it was not possible to appropriately examine the association between antihypertensive medication use during pregnancy and ASD/ADHD in offspring due to several limitations in the data. (See Section **11.3.1** below).

11.2.5 Limitations of Swedish National Register Studies

It is not possible to rule out the presence of residual confounding in observational studies. While it may have been reduced in the sibling-matched analysis, this method can only adjust for factors that are constant between pregnancies[229], therefore we cannot rule out the possibility of unmeasured confounding factors. Furthermore, outpatient data only started becoming available in 2001, meaning more severe cases of ASD/ADHD may have been overrepresented in our data[87], while less severe cases may have been assigned as controls. However, it is unlikely that this misclassification was differential based on exposure to preeclampsia (i.e. the proportion of cases and controls incorrectly assigned to the exposure group are similar), and thus may have biased results towards the null. Although, results of the sensitivity analyses attempting to investigate this further were not materially different from our main findings, suggesting the inclusion of less severe cases after 2001 may not have had a large impact on findings.

11.2.6 Limitations of Growing Up in Ireland Study

In contrast to the Swedish data studies, data on preeclampsia was self-reported 9-months post-delivery in the GUI study, therefore may have been subject to recall bias. Similarly, outcome data was reliant on the subjective evaluation of the child's mother, which may have introduced misclassification bias. However, it was not possible for

the child's mother to be influenced by our study hypothesis due to the secondary analysis nature of our study. Therefore, it is more likely that the misclassification was non-differential. Furthermore, loss to follow-up may have posed a threat to validity of findings. Previous evidence suggests children with behavioural disorders are more prone to loss to follow-up, which may have introduced selection bias[113]. Finally, not unlike the Swedish data study, we cannot rule out the possibility of residual confounding.

11.3 Future Directions

11.3.1 The Role of Antihypertensive Medications and Neurodevelopmental Outcomes

It is important for future research to explore the role of antihypertensive medications used during pregnancy in the development of neurodevelopmental outcomes. We initially made an attempt to examine the impact of antihypertensive medication using Swedish National Registers. However due to some major limitations in the data, it was deemed irresponsible to publish findings, and the analysis was omitted from the thesis. In sum, antihypertensive medication data were obtained from the Swedish Prescribed Drug Register, which on 1st July 2005, was expanded to include the PIN, allowing linkage to other registers[89]. The Prescribed Drug Register contains information on all dispensed prescribed drugs in primary care and outpatient care and coverage is almost 100% complete[90]. However, the register does not contain information on over-the-counter medications or medications used in hospital care[90]. All medications are classified according to Anatomical Therapeutic Chemical (ATC) classification system, and antihypertensive medication included in the drug register were labetalol

(ATC code: C07AG01), nifedipine (C08CA05), methyldopa (C02AB01) and hydralazine (C02DB02).

We included all live births between 1st July 2005 and 31st December 2010 in the analysis. Children were considered exposed if mothers purchased prescribed antihypertensive medication between date of conception and date of birth. As women diagnosed with preeclampsia are frequently managed as inpatients[2], they would receive medication within the hospital. However, as the Prescribed Drug Register does not contain information on medications used in hospital care, we compared the likelihood of ASD/ADHD among those exposed to antihypertensive medication for reasons other than preeclampsia (for example, chronic kidney disease, chronic hypertension and diabetes) and those exposed to neither preeclampsia nor antihypertensive medication. A major limitation of this analysis is confounding by indication as indications for antihypertensive medication for reasons other than preeclampsia may play a role in the development of neurodevelopmental outcomes. Furthermore, as we lacked data on medications used in hospital care, we could not be sure that medications prescribed for preeclampsia as inpatients corresponded to medications prescribed for other indications, concerning dose/timing etc.

If data on all medications used was available, it may be possible to address this research question. However, as confounding by indication would remain a concern, and can be a difficult issue to resolve in population-based epidemiological research[272, 273], it may be best to address this research question using animal models such as the RUPP model in rats, which mimics many physiological features of preeclampsia[227].

11.3.2 The Potential Introduction of Standardised Developmental Screening

Despite a general consensus that early identification and intervention can lead to improvements in long-term neurodevelopmental outcomes, a diagnosis is frequently not received until the child is attending school, meaning the window for early intervention has closed[228, 274]. However, research suggests that a stable diagnosis of ASD can be made as young as two years, while diagnosis and treatment of ADHD can begin at age four years, allowing earlier access to specialised services[134, 274]. Therefore, investigating the effectiveness of standardised developmental screening of infants previously exposed to preeclampsia is timely, and may allow for intervention and support at an earlier age, which in turn, may aid improvement of neurodevelopmental outcome[134].

However, there are arguments for and against the recommendation of introducing early screening for children exposed to preeclampsia. For example, the results observed in this thesis may not warrant the introduction of standardised screening and therefore, it is important not to instil unnecessary stress on women with a previous diagnosis of preeclampsia. Conversely, early screening is non-invasive, and can be a relatively straightforward process. A recent randomised controlled trial suggests that computer-automated screening can lead to improvements in ASD screening rates in a primary care setting[275]. Downs and colleagues developed a computer-based clinical decision support system called Child Health Improvement through Computer Automation (CHICA), with a built-in ASD decision support and compared it to using CHICA without ASD decision support. Screening rates in the intervention group increased from 0% at baseline to 68.4% in six months and to 100% in 24 months, while no significant increase in screening rates was observed in the control group[275]. However, as most children with a positive screening result will not have ASD,

resulting in a high false positive rate, referral may not be justified, and a cost-benefit analysis may be necessary before a more informed recommendation can be made[276].

11.4 Conclusion

Through rigorous investigation, including the use of multiple statistical modelling, and controlling for several potential confounding factors, the data presented in this thesis suggest that exposure to preeclampsia or preeclampsia and SGA combined (i.e. SGA baby exposed to preeclampsia) was associated with ASD and ADHD. The stronger association with preeclampsia and SGA combined than preeclampsia alone suggests that placental pathology may be a mechanism for the increased likelihood of ASD and ADHD. In addition to this, results of the current thesis suggest that preeclampsia may be associated with adverse neurodevelopmental outcomes across generations.

While we did not find strong evidence of associations between preeclampsia and child developmental and behavioural outcomes overall, exposure to preeclampsia was associated with an increased likelihood of subtle behavioural issues in the emotional and hyperactivity domain of the SDQ.

Overall, results of this thesis suggest an association between HDP and neurodevelopmental outcomes in offspring. However, it is important to note that it is not possible to rule out the presence of residual confounding in observational studies. Furthermore, the associations observed in this thesis might lack specificity, as preeclampsia (and preeclampsia and SGA combined) may be associated with several neurodevelopmental outcomes. Therefore, in conclusion, preeclampsia may in fact increase the likelihood of poor neurodevelopmental outcome in general, with the specificity of outcome being determined by underlying genetic risk factors[200].

APPENDICES

Appendix 1: Search strategy for identifying relevant studies in the systematic review

Search terms
e.g. For PubMed (1946 - June, 7th 2017) LIMIT: humans, English language
1. pre eclampsia
2. preeclampsia
3. pre-eclampsia
4. gestational hypertension
5. hypertensive pregnancy disorder
6. hypertensive disorders of pregnancy
7. pregnancy induced hypertension
8. pregnancy-induced hypertension
9. pregnancy hypertension
10. toxaemia
11. toxemia
12. maternal metabolic
13. [#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12]
14. autism
15. autism spectrum
16. autistic
17. autism spectrum disorders
18. autism Spectrum Disorder
19. autistic spectrum disorders
20. autistic Spectrum Disorder
21. asperger
22. asperger's
23. asperger's Syndrome
24. autistic Spectrum
25. pervasive developmental disorder
26. pervasive developmental disorders
27. disintegrative disorder
28. rett syndrome
29. attention deficit disorder
30. ADD
31. ADHD
32. attention-deficit
33. attention-deficit/hyperactivity disorder
34. attention-deficit hyperactivity disorder
35. attention-deficit-hyperactivity disorder
36. hyperactivity disorder
37. hyperactiv*
38. overactive*
39. inattent*
40. hyperkinetic disorders
41. hyperkinet*
42. neurodevelopment
43. specific learning disorder
44. learning disorder
45. intellectual disability
46. mental retardation
47. communication disorder
48. motor disorder
49. conduct disorder
50. IQ
51. reading age

52.	school performance
53.	[#14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52]
54.	[#13 and #53]

Appendix 2: Bias classification tool to appraise quality of studies included in the systematic review

Bias	NR	Minimal	Low	Moderate	High
Selection	□	□ Consectutive unselected population □ Sample selected from general population rather than a select group □ Eligibility criteria explained □ Rational for case and control selection explained □ Follow up or assessment time explained	□ Sample selected from large population but selection criteria not defined □ A select group of population (based on race, ethnicity, residence, etc.) studied	□ Sample selection ambiguous but sample may be representative □ Eligibility criteria not explained □ Rationale for case and controls not explained □ Follow up or assessment time not explained	□ Sample selection ambiguous and sample likely not representative □ Comparative groups differ in baseline characteristics □ A very select population studied making it difficult to generalise findings
Exposure	□	□ Direct questioning (interview) or completion of survey by mother at the time of exposure or close to the time of exposure □ Direct measurment of exposure (laboratory) □ Exposure from the chart	□ Assessment of exposure from a dataset □ Indirect assessment (postal survey, mailed questionnaire) □ Recall < 1 year after birth	□ Recall 1-5 years after birth □ Extrapolating data from population exposure sample (with some assumptions) and not direct assessment at any time	□ Recall >5 years after birth □ Indirect method of assessment (obtaining data from others and not from mother or father)
Outcome	□	□ Assessment from hospital record, birth certificate or from direct question to mother about outcome	□ Assessment from administrative database	□ Assessment from "close-ended" questions (Did you have an ectopic pregnancy?)	□ Assessment from non-validated sources or generic estimate from overall population
Confounding	□	□ Assessed for common confounders	□ Only certain confounders assessed	□ Not assessed for confounders	
Analytical	□	□ Analyses appropriate for type of sample (if matched: paired t test, McNemar) □ Analytical method accounted for sampling strategy in cross sectional study □ Sample size calculation performed and adequate sample studied	□ Analyses not accounting for common statistical adjustment (e.g. multiple analyses e.g. Bonferroni) when appropriate □ Sample size calculation not	□ Sample size estimation unclear or only sub-sample of eligible patients studied	□ Analyses inappropriate for type of sample/study

			performed, but all available eligible patients studied □ Sample size calculated and reasons for not meeting sample size given		
Attrition	□	□ None or <10% attrition and reasons for loss of follow up explained □ All subjects from initiation of study to final outcome assessment were accounted for	□ <10% attrition AND reasons for loss of follow up not explained □ 11-20% attrition, reasons for loss of follow up explained	□ 11-20% attrition but reasons for loss of follow up not explained □ >20% attrition but reasons for loss of follow up explained □ All subjects from initiation of study to final outcome assessment not accounted for	□ >20% attrition, reasons for loss of follow up not explained

Appendix 3: Characteristics of ASD studies included in the systematic review

Study	Data source	Study design	Region, study period	Sample size and prevalence of exposure	Diagnosis of HDP	Outcome	Assessment method	Confounders adjusted	Matching factors	Confounders identified?
Curran et al, 2018[21]	Millennium Cohort study	Cohort	UK 2000-01	HDP 983, No HDP 12115 HDP= 7.5%	Doctor-diagnosed self-reported HDP	ASD	Maternal-reported	Smoking during pregnancy, birth order, poverty, maternal ethnicity, age, education, depression, BMI, longstanding diabetes, longstanding HT	n/a	Literature
Walker et al, 2015[13]	CHARGE study	Case-control	California 2003-11	ASD 517, Controls 350	PE from medical records or maternal self-reporting in telephone interview. (Diagnostic criteria NR)	ASD	Previous ASD diagnoses were examined using the ADOS and the primary caregiver was administered the ADI-R	Maternal educational level, parity, pre-pregnancy obesity	Age, sex, broad geographic regions within the study catchment areas	Literature and DAG
Polo-Kantola et al, 2014[35]	National registry data	Case-control	Finland 1990-2005	ASD 1036, Controls 4132	Maternal HT: PE and/or PIH from MBR: BP >140/90	ASD	ICD-10	Maternal age, maternal smoking during pregnancy, number of previous births, maternal psychiatric history	Sex, date of birth, place of birth	Literature
Langridge et al, 2013[36]	MNS, Registrar General's birth and death reg-istrations	Cohort	Western Australia 1984-99	ASD without ID 452, no ASD 376529	Pregnancy hypertension (PE and essential	ASD	DSM-IIIR, DSM-IV, DSM-IV-TR	Birth year, maternal and pregnancy conditions (maternal	n/a	NR

				Prevalence of HDP: NR	hypertension) from MNS. (Diagnostic criteria NR)			diabetes, threatened abortion, asthma, UTI during pregnancy, placenta praevia, placenta abruption, other antepartum haemorrhage), socio-demographics (parity, maternal and paternal age group, maternal ethnicity, community-level socioeconomic status and community accessibility/remoteness), labour and delivery factors (preterm type, mode of delivery, breech, any complication of labour or delivery), neonatal outcomes (infant gender, resuscitation required at birth, percentage of optimal birthweight and		

								head circum-ference)		
Mrozek-Budzyn et al, 2013[12]	Psychiatric outpatient clinic for children	Case-control	Poland 2006-07	Cases 96, Controls 192	PE and chronic HT from medical records or self-reporting. (Diagnostic criteria NR)	Childhood or atypical autism	ICD-10	No	Year of birth, sex and general prac-titioners	Only factors associated with ASD in univariate model were included in multivariate model
Nath et al, 2012[11]	Neuro-development and Early Intervention Clinic	Case-control	India 2012	Cases 31, Controls 100	PIH: Self-reported	ASD	DSM IV TR	No	Age	NR
Lyall et al, 2012[9]	Nurses' Health Study II	Cohort	United States 1989-2005	Total 66445, Toxemia 5968, Pregnancy-related HBP 5884 Toxemia= 9% HBP= 8.9%	Toxemia and pregnancy related HBP self-reported in question-naire	ASD	Maternal-reporting	Race, marital status, income, spouse education, nurse's age at baseline, age at first birth, parity	n/a	Literature
Krakowiak et al, 2012[10]	CHARGE study	Case-control	Cali-fornia, 2003-10	Cases 517, Controls 315	Hypertension (chronic, gestational or PE) from medical records or structured interview with the mother. (Diagnostic criteria NR)	ASD	ADI-R and ADOS	Mother's age at delivery, race/ethnicity, education level, delivery payer, calendar time	Age, gender, and regional centre catchment area	DAG
Dodds et al, 2011[50]	Admin-istrative Health Databases	Cohort	Nova Scotia, Canada 1990-2002	PIH 11836, No PIH 117897 PIH= 9.1%	PIH from Perinatal Database: ICD-9 and ICD-10	ASD	ICD-9 and ICD-10	No	n/a	Only factors associated with ASD in univariate model were included in multivariate model

Burstyn et al, 2010[31]	Provincial delivery records and physician billing data	Cohort	Alberta, Canada 1998-2004	PE 2774, No PE 213568 PE= 1.3%	PE from APHP delivery records. (Diagnostic criteria NR)	ASD	ICD-9	Maternal age, maternal weight, maternal height, pre-pregnancy diabetes, gestational diabetes, bleeding, smoking, poor weight gain, parity, mother's SES, presentation (breech etc.), type of labour, caesarean section, gestational age, birthweight, APGAR at 1 min and 5 mins, infant sex, birth year.	n/a	NR
Mann et al, 2010[30]	Birth certificate and Medicaid billing records	Cohort	South Carolina 1996-2002	PE 5531, No PE 82146 PE= 6.3%	PE/ eclampsia from billing records for Medicaid-eligible women, ICD-9	ASD	ICD-9 from Medicaid billing records or children receiving services from the South Carolina DDSN for autism	Maternal age, race, alcohol use, educational attainment, year of birth, child's sex, and diagnosis with a high risk condition (alcohol use, tobacco use, down syndrome, fragile X syndrome, brain anomaly) and birthweight	n/a	NR
Bilder et al, 2009[49]	Birth certificate records and ADDM	Case-control	Utah, US 1994-2002	Cases 132, Controls 13200	Chronic and PIH from birth certificate records.	ASD	DSM-IV-TR from ADDM	No	Gender and birth year	NR

					(Diagnostic criteria NR)					
Buchmayer et al, 2009[14]	Swedish MBR and Hospital Discharge Register	Case-control	Sweden 1987-2002	Cases 1216, Controls 6080	PE and gestational HT from the MBR: ICD-9 and ICD-10	Autistic disorders	ICD-9 and ICD-10	Parity, previous miscarriage, childless years, any maternal infection during pregnancy, season of delivery, diabetes mellitus, maternal age, smoking, maternal country of birth, whether the mother lived with the father, maternal schizophrenia	Age, gender, birth year, and birth hospital	Literature
Larsson et al, 2005[148]	Danish PCR, Danish MBR and IDA	Case-control	Denmark 1978-90	Cases 698, Controls 17450	PE from MBR. (Diagnostic criteria NR)	Autism	ICD-8 and ICD-10 from PCR	No (information on PE available from 1978-90 only)	Gender, birth year and age	NR
Glasson et al, 2004[34]	MCHRDB	Case-control	Western Australia 1980-95	Cases 314, Controls 1313	PE: ICD-9	Autism	DSM	No	Sex	NR
Hultman et al, 2002[15]	Swedish MBR and In-patient Register	Case-control	Sweden 1974-93	Cases 408, Controls 2040	HDP from Medical Birth Register: ICD-8 and ICD-9	Infantile autism	ICD-9 - Discharged from a Swedish psychiatric or general hospital with a main diagnosis of infantile autism	Maternal age, parity, smoking during pregnancy, mother's country of birth, diabetes, pregnancy bleeding, mode of delivery, season of birth, gestational age, birthweight for gestational age, Apgar score at 5	Sex, year, and hospital of birth	NR

								minutes, congenital malformations		
Eaton et al, 2001[62]	Danish MBR and Danish PCR	Case-control	Denmark 1973-93	Cases 116, Controls 102905	Eclampsia from MBR. (Diagnostic criteria NR)	Autism	ICD from PCR	Gender and year of birth	NR	Only variables significantly associated with outcome included in multivariate analysis
Matsuishi et al, 1999[33]	NICU survivors of St. Mary's Hospital, Kurume	Case-control	Kurume, Japan 1983-87	Cases 18, Controls 214	Toxemia. (Diagnostic criteria NR)	Autistic disorder	DSM-III-R	No	NR	NR
Mason-Brothers et al, 1990[32]	Survey data and medical records	Case-control	Utah, US 1965-84	Cases 225, Controls 60	Toxemia from medical records. (Diagnostic criteria NR)	Autism	DSM-III from survey	No	Sibling	NR
Deykin et al, 1980[147]	Referral agencies and medical records and interview data	Case-control	Mas-sachusetts, US 1975-77	Cases 118, Controls 246	Toxemia from medical records and interview data. (Diagnostic criteria NR)	Autism	≥1 symptoms of impaired relatedness to the environment, stereopathy and impaired language development	Birth order	Sibling	Excess of first born among cases

ASD=autism spectrum disorder. HDP=hypertensive disorder of pregnancy. BMI=body mass index. HT=hypertension. n/a=not applicable. CHARGE=Childhood Autism Risks from Genetics and the Environment. PE=preeclampsia. NR=not reported. ADOS=Autism Diagnostic Observation Schedule. ADI-R=Autism Diagnostic Interview, Revised. DAG=directed acyclic graph. PIH=pregnancy-induced hypertension. MBR=Medical Birth Register. BP=blood pressure. ICD=International Classification of Disease. MNS=Midwives' Notification System. ID=intellectual disability. DSM=Diagnostic and Statistical Manual of Mental Disorders. UTI=urinary tract infection. HBP=high blood pressure. APHP=Alberta Perinatal Health Program. SES=socioeconomic status. DDSN=Department of Disabilities and Special Needs. ADDM=Autism Developmental Disabilities Monitoring Network. PCR=Psychiatric Central Register. IDA=Integrated Database for Longitudinal Labour Market Research. MCHRDB=Maternal and Child Health Research Database. NICU=neonatal intensive care unit.

Appendix 4: Characteristics of ADHD studies included in the systematic review

Study	Data source	Study design	Region, study period	Sample size and prevalence of exposure	Diagnosis of HDP	Outcome	Assessment method	Confounders adjusted	Matching factors	Confounders identified?
Böhm et al, 2017[22]	Millennium Cohort study	Cohort	United Kingdom 2001-08	HDP 1069, No HDP 12432 HDP= 7.9%	Self-reported HDP	ADHD	Maternal-reported	Alcohol during pregnancy, maternal education, maternal depression, maternal age, poverty status	n/a	Literature
Silva et al, 2014[39]	MNS and MODDS system	Case-control	Western Australia 1981- 2003	Cases 12991, Controls 30071	PE from MNS system. (Diagnostic criteria NR)	ADHD	DSM-IV or ICD-10 Data extracted from MODDS on children and young adults dispensed stimulant medication	Marital status, parity, smoking, complications of pregnancy, onset of labor, augmentation of labor, complications of labor, type of delivery, child characteristics (gestational age, birthweight, average/small/ large for gestational age) maternal age, Apgar at five mins.	Year of birth, gender, and socio-economic status	Available from MNS for data analysis
Cak and Gokler, 2013[48]	NICU hospital records	Cohort	Turkey 2003-08	Total 106, PE 16, HT 22 HT= 20.8% PE= 15.1%	HT and PE: Self-reported and NICU records. (Diagnostic criteria NR)	ADHD	K-SADS-PL according to DSM-IV	No	n/a	NR

Getahun et al, 2013[40]	KPSC medical records	Case-control	Southern California 1995-2010	Cases 13613, Controls 68065	PE:ICD-9-CM	ADHD	Clinical diagnosis of ADHD using ICD-9-CM on at least 2 separate visits or a diagnosis on 1 visit and at least 2 refills of ADHD-specific medications	Maternal age, education, smoking during pregnancy, parity, prenatal care, household income, psychosocial disorder during pregnancy, child race/ethnicity, and gender	Age at diagnosis	"Chosen *a priori"*
Golmirzaei et al, 2013[42]	Cluster sampling of preschool children	Case-control	Southern Iran 2012	Cases 208, Controls 196	PE self-reported in questionnaire	ADHD	Conners' parents and teachers rating scale and interview by a child and adolescent psychiatrist using DSM-IV criteria	No	Age	NR
Amiri et al, 2012[47]	Child and Adolescent Psych-iatric Clinics. Controls from primary school students	Case-control	Tabriz, Iran 2009	Cases 164, Controls 166	PE self-reported and medical records when possible. (Diagnostic criteria NR)	ADHD	The ADHD Rating Scale-Parent Version questionnaire according to DSM-IV-TR criteria and K-SADS according to DSM-III-R and DSM-IV	No	Age	NR
Halmoy et al, 2012[45]	MBR of Norway	Cohort	Norway 1967-87	Total 1172396, Chronic HT 1570, PE 28495 HT= 0.13% PE= 2.4%	Chronic hyper-tension and PE from the MBR. (Diagnostic criteria NR)	ADHD	Adult ADHD patients who were approved for stimulant treatment in Norway during 1997–2005,	Year of birth, parity, age of mother at birth, educational level of mother and marital status of mother	Born in the same time period	NR

							according to ICD-10 criteria, modified to be comparable to DSM-IV			
Ketzer et al, 2012[46]	12 public schools in Porto Alegre, Brazil and ADHD outpatient programme	Case-control	Brazil 2001-07	Cases 124, Controls 124	PE/ eclampsia self-reported and medical records when possible. (Diagnostic criteria NR)	ADHD	K-SADS-E and DSM-IV criteria	Agoraphobia (anxiety disorder), maternal ADHD and cigarettes/day during pregnancy	Age, gender	Literature
Gustafsson and Källén, 2011[44]	Swedish MBR and Department of Child and Adolescent Psychiatry Register	Cohort	Sweden 1986-2006	PE 888, No PE 31124 PE= 2.8%	PE from MBR. (Diagnostic criteria NR)	ADHD	DSM-III-R11 before 1994 and DSM-IV12 from 1994 onwards	No	n/a	Only variables with p<0.2 included in multivariate analysis
Mann and McDermott, 2011[41]	Medicaid billing records	Cohort	South Carolina 1996-2002	PE 4674, No PE 80047 PE= 5.5%	PE/ eclampsia: ICD-9	ADHD	Diagnosed with ADHD using ICD-9 by at least two different providers	GU infection, infant race, maternal age and education, alcohol and tobacco use, infant sex, birthweight, and oldest age in Medicaid	n/a	Literature

ADHD=attention deficit/hyperactivity disorder. HDP=hypertensive disorder of pregnancy. n/a=not applicable. MNS=Midwives' Notification System. MODDS=Monitoring of Drugs of Dependence System. PE=preeclampsia. NR=not reported. DSM=Diagnostic and Statistical Manual of Mental Disorders. ICD=International Classification of Disease. ICD-9-CM=International Classification of Diseases, Ninth Revision, Clinical Modification. NICU=neonatal intensive care unit. HT=hypertension. K-SADS-PL=Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version. KPSC=Kaiser Permanente Southern California. K-SADS-E=Schedule for Affective Disorders and Schizophrenia for School Age Children-Epidemiological Version. MBR=Medical Birth Registry. GU=genitourinary.

Appendix 5: Characteristics and main findings of other neurodevelopmental outcome studies included in the systematic review

Summary of HDP and cognitive functioning/developmental delay studies								
Preeclampsia								
Study	**Population**	**Study design**	**Region, study period**	**Sample size**	**Diagnosis of HDP**	**Outcome measure**	**Assessment method**	**Main findings**
Warshafsky et al, 2016[255]	Offspring, age 1-5 years	Cohort	Kingston and Ottawa, Canada 2003-09	PE 95, No PE 140	Severe PE: BP >140/90 mm Hg and proteinuria >300 mg/24 hours or ≥1+ on repeat dipstick	Neurodevelopmental performance	Failure of Ages and Stages Questionnaire	Severe PE v NT: No significant associations OR and 95% CI: Year 1 follow-up: 0.90 (0.24 to 3.34) Year 2 follow-up: 0.63 (0.19 to 2.09) Year 3 follow-up: 2.31 (0.63 to 8.53)
Walker et al, 2015[13]	Offspring, aged 24-60 months	Case-control	20 Californian counties, 2003-11	Developmental delay 138, typical development 277	PE: medical records	Development delay	Vineland Adaptive Behaviour Scales, Mullen Scales of Early Learning, Social Communication Questionnaire	PE (medical records only) v NT: No significant association OR and 95% CI: 1.82 (0.72, 4.64)
Heikura et al, 2013[277]	Offspring, age 11.5 years	Cohort	Oulu and Lapland, Northern Finland 1985-86	PE 267, NT 6897	PE: BP ≥140/90mm Hg and proteinuria	Mild cognitive limitations	IQ between 50 and 85 based on standardised psychometric tests (eg. WISC-R)	PE v NT: No significant association OR and 95% CI: 1.2 (0.5, 2.8)
Tuovinen et al, 2013[278]	Offspring, 70 years later	Cohort	Helsinki, Finland 1934-44	PE 31, NT 553	PE: proteinuria and SBP ≥140mm Hg or DBP ≥90mm Hg	Self-reported cognitive impairment	CFQ and DEX	PE associated with significantly more complaints of cognitive functioning (MD for total score 0.45 (0.02, 0.87) and more complaints of dysexecutive functioning, but not significant 0.31 (-0.11, 0.73)

Love et al, 2012[279]	All children born to mothers in Aberdeen city between 1995-2008	Cohort	Aberdeen, Scotland 1995-2008	PE 1774, NT 23334	PE: Davey and MacGillivray's classification of HDP	Congenital abnormality, cerebral palsy, autism, ADHD, developmental delay, communication difficulties/learning difficulties and other	Record in SNS	NT v PE: No significant association OR and 95% CI: 0.80 (0.60, 1.07)
Whitehouse et al, 2012[280]	Offspring, age 10 years	Cohort	Western Australia 1989-91	PE 34, NT 1076	PE: gestational HT with proteinuria of ≥300mg/24hr.	Neurocognitive development	PPVT-R and RCPM	PE not associated with lower PPVT-R scores (MD for total score -3.35 (-8.41, 1.35) or lower RCPM scores (MD for total score -1.82 (-12.59, 8.95)
Ehrenstein et al, 2009[281]	Men born in 1978-83	Cohort	Northern Denmark 1978-83	PE 604, NT 16566	PE: BP >140/90mm Hg in second half of pregnancy and de novo proteinuria (>0.3g over 24hrs) or edema	Adult cognitive function	BPP group intelligence test	PE v NT: PE associated with increased odds of low cognitive function PR and 95% CI: 1.32 (1.08, 1.62)
Eaton et al, 2001[62]	Offspring, age <15 years	Case-control	Denmark 1973-93	Learning disorders 580, reference population 102905	Eclampsia from Medical Birth Register	Learning disorders	ICD8	Eclampsia v NT: No significant association RR: 0.9
Seidman et al, 1991[282]	Offspring, age 17 years	Cohort	Jerusalem, Israel 1964-71	PE 428, No PE 33117	PE: After 24 weeks gestation, SBP ≥140mm Hg or DBP ≥90mm Hg or rise in BP of ≥30/15mm Hg (two readings ≥6hrs apart) or proteinuria or oedema of the face and arms or any combination of 2 or more	Intelligence score	Verbal Otis test and nonverbal matrices test transformed into values that correlate with the WAIS	No difference in mean IQ test scores between PE and non PE: mean 109.3 (1.2) v 110.9 (0.1)

Barker and Edwards, 1967 (283)	Offspring, age 11 years	Cohort	Birmingham, UK 1950-54	Toxemia 3321, No toxaemia 42329	Toxemia: HT or albuminuria during pregnancy	Verbal reasoning	Eleven-plus	Toxemia associated with lower verbal reasoning within sibpairs (MD in unaffected sibs in subsequent birth -0.7 and preceding birth -2.2)
Preeclampsia (specific population)								
Study	**Population**	**Study design**	**Region, study period**	**Sample size**	**Diagnosis of HDP**	**Outcome measure**	**Assessment method**	**Main findings**
Johnson et al, 2015(69)	Late and moderately preterm infants	Cohort	East Midlands, United Kingdom 2009-10	638 completed questionnaire at follow-up	PE: self-reported	Cognitive development at age 2	PARCA-R	PE associated with increased risk of cognitive impairment. RR and 95% CI: 2.51 (1.33, 4.70)
Morsing and Maršál, 2014(61)	IUGR and very preterm birth	Cohort	Lund University Hospital, Sweden 1998-2004	PE 11, No PE 23	PE: >90mm Hg on 2 or more occasions and proteinuria >300mg/L	Cognitive impairment	Wechsler scales	IUGR infants exposed to PE had significantly lower full-scale IQ compared to IUGR infants unexposed to PE: PE: 70.1 (±19) Non PE: 83.3 (±14)
Leitner et al, 2012(284)	IUGR infants	Cohort	Lis Maternity Hospital, Israel 1992-2002	PE 17, NT 78	PE: SBP ≥140mm Hg or DBP ≥90mm Hg developing after 20 weeks gestation with proteinuria >0.3g in 24/hr urine sample or +2 in dipstick urine test, without history of previous HT	IQ and academic achievement	WISC-R95 two-test short form and Kauffman Assessment Battery for Children	No significant differences observed between the groups

Leversen et al, 2011[149]	Children born extremely preterm (22-27 weeks gestation)	Cohort	Norway 1999-2000	PE 73, No PE 233	PE: Medical Birth Registry of Norway	Cognitive function at age 5	Wechsler Preschool and Primary Scale of Intelligence-Revised	PE associated with lower full-scale IQ MD -7.7 (-12.7, -2.7)
Schlapbach et al, 2010[64]	Preterm infants <32 weeks gestation	Cohort	University Hospital Zurich, Switzerland 2002-05	PE 33, No PE 33	PE: proteinuria >300mg/d and DBP >90mm Hg in two measurements ≥4 hrs apart after 20th week gestation and regressing after delivery and/or acute spiral artery atherosis on placental histology or placental bed biopsy	Adverse neurodevelopmental outcome	Bayley Scales of Infant Development II: MDI<70 and/or PDI<70	No association: PE v No PE: OR and 95% CI: 1.36 (0.46, 4.04)
Spinillo et al, 2009[66]	Preterm infants (24-33 weeks gestation)	Cohort	Pavia, Italy 1990-2004	PE 185, No PE 569	PE: DBP ≥110mm Hg or ≥90mm Hg in two consecutive measures at any time during pregnancy and proteinuria ≥300mg/day	MDI	Bayley Scales of Infant Development II	PE associated with reduced risk of impairment. OR and 95% CI: 0.52 (0.32, 0.85)
Silveira et al, 2007[68]	VLBW infants	Cohort	Hospital de Clínicas de Porto Alegre, Brazil 2003-05	PE 40, No PE 46	PE: SBP ≥140mm Hg and/or DBP ≥90mm Hg developing after 20 weeks gestation with proteinuria >300mg in 24/hr urine sample, without history of previous HT or renal disease	MDI at 12 and 18 months	Bayley Scales of Infant Development II	Mean MDI scores not significantly different. At 12 months: PE: 79.6 (±0.44) No PE: 79 (±0.47) At 18 months: PE: 82.9 (±0.45) No PE: 81.1 (±0.7)

Cheng et al, 2004[57]	VLBW infants <32 weeks gestation	Cohort	Taiwan 1997-99	PE 28, No PE 61	PE: DBP of 110mm Hg once or DBP of ≥90mm Hg twice and proteinuria of ≥300mg in 24/hr	MDI	Bayley Scales of Infant Development II	Median MDI score significantly lower for PE compared to non-PE: PE: 72 (49-116) Non-PE: 86 (49-114) p=0.04
Many et al, 2003[60]	Children born growth restricted	Cohort	Lis Maternity Hospital, Israel 1992-93	PE 11, No PE 64	PE: persistent BP ≥140/90mm Hg with proteinuria of 100mg/dL by random urine analysis or >500mg in 24hr urine collection	Cognitive assessment	Standford Binnet-IQ	Growth restricted infants exposed to PE had significantly lower IQ scores compared to unexposed growth restricted: PE: 85.5 (±16) Non PE: 96.9 (±18)
Szymonowicz et al, 1987[58]	VLBW infants	Cohort	Australia 1982-84	PE 35, No PE 35	Severe PE: >140/90mm Hg, persistent proteinuria with UTI and generalised oedema <32 weeks gestation	MDI	Bayley Scales of Infant Development II	PE associated with significantly lower mean MDI PE: 94 No PE: 106
Other HDP								
Study	**Population**	**Study design**	**Region, study period**	**Sample size**	**Diagnosis of HDP**	**Outcome measure**	**Assessment method**	**Main findings**
Heikura et al, 2013[277]	Offspring, age 11.5 years	Cohort	Oulu and Lapland, Northern Finland 1985-86	GH 443, Chronic HT or superimposed PE 564, NT 6897	GH: BP ≥140/90mm Hg Chronic HT or superimposed PE: already using anti-HT medication at the beginning of pregnancy or having blood pressure ≥140/90 mmHg before week 20	Mild cognitive limitations	IQ between 50 and 85 based on standardised psychometric tests (eg. WISC-R)	GH v NT: GH associated with increased odds of mild cognitive limitations OR and 95% CI: 2.4 (1.4, 3.9). Chronic HT v NT: No significant association OR and 95% CI: 1.4 (0.8, 2.5)

					classified as having chronic hypertension. With a positive urinary dip-stick test (≥0.3 g/L) indicated proteinuria			
Tuovinen et al, 2013[278]	Offspring, 70 years later	Cohort	Helsinki, Finland 1934-44	HT 292, NT 553	Gestational and chronic HT: SBP ≥140mm Hg or DBP ≥90mm Hg at <20 weeks gestation, without proteinuria	Self-reported cognitive impairment	CFQ and DEX	HT associated with more complaints of cognitive functioning (MD for total score 0.12 (-0.04, 0.27) and more complaints of dysexecutive functioning, 0.07 (-0.08, 0.22), but neither result significant
Krakowiak et al, 2012[10]	Offspring, age 2-5 years	Case-control	California 2003-10	Developmental delay 64, typical development 172	HT (with or without PE) self-reported or medical records	Developmental delay	Vineland Adaptive Behaviour Scales, Mullen Scales of Early Learning, Social Communication Questionnaire	HT v NT: No significant association OR and 95% CI: 3.58 (0.93, 13.78)
Love et al, 2012[279]	All children born to mothers in Aberdeen city between 1995-2008	Cohort	Aberdeen, Scotland 1995-2008	GH 4092, NT 23334	GH: Davey and MacGillivray's classification of HDP	Congenital abnormality, cerebral palsy, autism, ADHD, developmental delay, communication difficulties/learning difficulties and other	Record in SNS	NT v GH: No significant association OR and 95% CI: 1.16 (0.99, 1.36)
Tuovinen et al, 2012[285]	Men (military service), age 20 years	Cohort	Helsinki, Finland 1934-44	HT 449, NT 747	HDP: BP ≥140/90mm Hg at any time during pregnancy	Intellectual abilities at military service	Finnish Defence Forces Basic Ability Test	MD and 95% CI in total intellectual abilities score: -0.12 (-0.24, -0.00)

Tuovinen et al, 2012[286]	Men (military service), age 20 and 69 years	Cohort	Helsinki, Finland 1934-44	HT 146, NT 252	HDP: BP ≥140/90mm Hg at any time during pregnancy	Intellectual abilities at military service	Finnish Defence Forces Basic Ability Test	Men born to HT mothers scored lower on tests: MD and 95% CI in total intellectual abilities score at age 69: -4.36 (-7.55,-1.17) and in decline in total cognitive ability -2.88 (-5.06,-0.70)
Whitehouse et al, 2012[280]	Offspring, age 10 years	Cohort	Western Australia 1989-91	PE 279, NT 1076	Gestational HT: SBP ≥140mm Hg or DBP ≥90mm Hg in women normotensive at <24 weeks gestation	Neurocognitive development	PPVT-R and RCPM	HT associated with lower PPVT-R scores (MD for total score -1.71 (-3.39, -0.03) but not associated with lower RCPM scores (MD for total score 0.15 (-3.60, 3.90)
Ehrenstein et al, 2009[281]	Men born in 1978-83	Cohort	Northern Denmark 1978-83	GH 287, NT 16566	GH: BP >140/90mm Hg in second half of pregnancy	Adult cognitive function	BPP group intelligence test	GH v NT: GH associated with increased odds of low cognitive function PR and 95% CI: 1.34 (1.01, 1.77)
Lawlor et al, 2005[287]	Offspring, age 7, 9 and 11 years	Cohort	Aberdeen, Scotland 1950-56	PIH 1977, No PIH 9702	PIH: PE or GH from Aberdeen Maternal and Neonatal Database	Childhood intelligence	Age 7: Moray House Picture Intelligence 1&2. Age 9: Schonell and Adams Essential Intelligence form A&B. Age 11: battery of Moray House Tests (2 verbal reasoning, arithmetic and English)	PIH v No PIH: MD in IQ points and 95% CI: 2.35 (1.56, 3.14) Results attenuated towards the null when adjusted for parental characteristics

Other HDP (specific population)								
Study	**Population**	**Study design**	**Region, study period**	**Sample size**	**Diagnosis of HDP**	**Outcome measure**	**Assessment method**	**Main findings**
Leitner et al, 2012[284]	IUGR infants	Cohort	Lis Maternity Hospital, Israel 1992-2002	GH 25, NT 78	GH: SBP ≥140mm Hg or DBP ≥90mm Hg developing after 20 weeks gestation without history of previous HT	IQ and academic achievement	WISC-R95 two-test short form and Kauffman Assessment Battery for Children	No significant differences observed between the groups
Many et al, 2005[288]	Children born with severe growth restriction	Cohort	Israel Date: NR	HDP 22, No HDP 70	HDP: NR	IQ at age 6	Wechsler Preschool and Primary Scale of Intelligence	No significant difference in mean IQ HDP: 106 (±11) No HDP: 101 (±14)
McCowan et al, 2002[289]	SGA children (birthweight <10th centile)	Cohort	New Zealand 1993-97	HDP 88, No HDP 132	HDP: BP ≥140/90mm Hg with an increase of ≥15mm Hg in DBP on 2 occasions >4hrs apart after 20 weeks gestation and/or proteinuria of >300mg/24hr and/or at least +2 proteinuria on repeated testing with urine dipsticks, without UTI	MDI	Bayley Scales of Infant Development II	HDP associated with higher MDI scores. Mean MDI: HDP: 98.6 No HDP: 93.7

Gray et al, 1998[290]	Very preterm infants (24-32 weeks gestation)	Cohort	Mater Mother's Hospital, Brisbane, Australia 1992-93	Maternal HT 107, No maternal HT 107	Maternal HT: Australasian Society for the Study of Hypertension in Pregnancy	Developmental delay	Griffith's Infant Ability Scale	Maternal HT not associated with developmental delay OR and 95% CI: 1.33 (0.61, 2.99)
Spinillo et al, 1994[291]	Preterm infants (24-35 weeks gestation)	Cohort	Italy 1986-90	HDP 92, No HDP 184	HDP: Davey and MacGillivray	Minor neurodevelopmental impairment	Bayley Scale of Infant Development	HDP associated with increased risk of minor impairment OR and 95% CI: 3.1 (1.41, 6.88)
Winer et al, 1982[292]	SGA infants (<10th centile)	Cohort	USA 1973-76	HDP 20, No HDP 35	HDP: American College of Obstetricians and Gynaecologists	Verbal IQ, performance IQ and full-scale IQ	Wechsler Preschool and Primary Scales of Intelligence or WISC-R and Raven's Coloured Progressive Matrices	HDP associated with higher verbal IQ score Mean and SD: HDP: 105.75 (13.50) No HDP: 93.68 (12.84) No significant differences observed for performance or full-scale IQ

Summary of HDP and other behavioral outcome studies

Preeclampsia

Study	Population	Study design	Region, study period	Sample size	Diagnosis of HDP	Outcome measure	Assessment method	Main findings
Robinson et al, 2009[67]	Offspring at age 2, 5, 8, 10 and 14 years	Cohort	Western Australia 1989-91	PE: 80 NT: 2119	PE: BP ≥140/90mm Hg after 24 weeks gestation and	Behavioural problems in childhood and adolescence	CBCL	No significant association between PE and overall

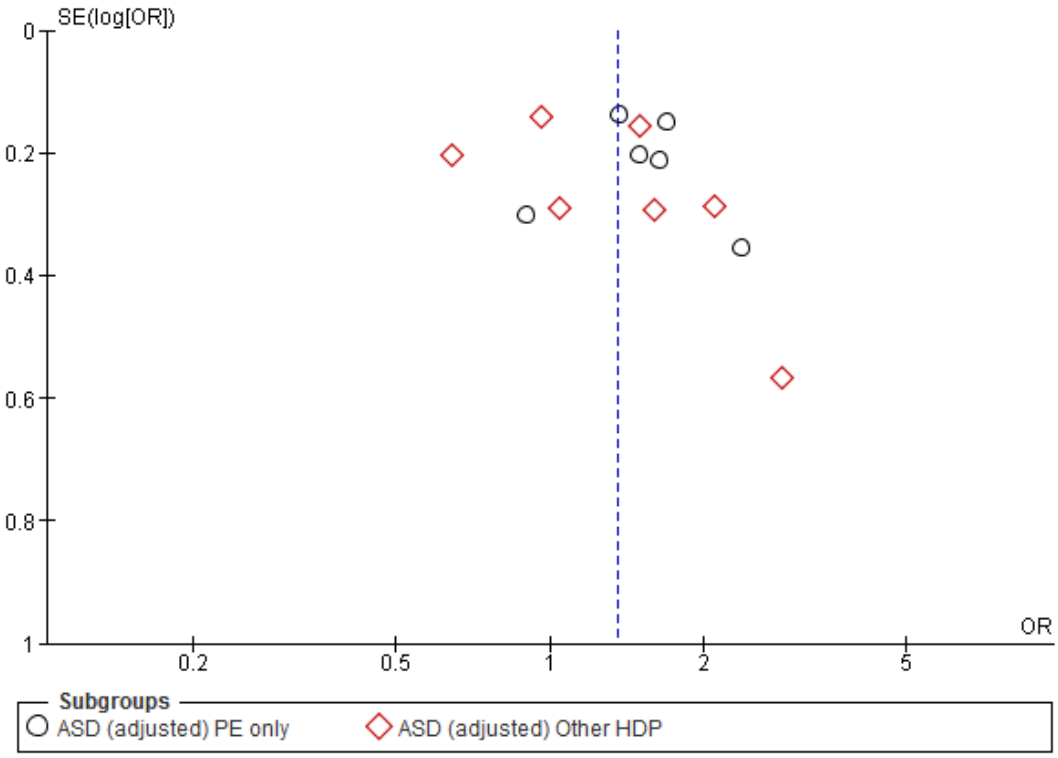
					proteinuria (≥0.3g/24hr)			behavioural problems. Protective relationship observed between PE and internalising behaviour problems at age 5 and 8. OR and 95% CI: 0.22 (0.05, 0.97) 0.33 (0.11, 0.98)
Wu et al, 2009[293]	All singletons born in Denmark between 1978 and 2004	Cohort	Denmark 1978-2004	PE 46384, No PE 1499059	PE: ICD8 and ICD10	Disease specific hospitalisations	Hospitalisation as a result of mental and behavioural disorders	PE not associated with increased risk of hospitalisation IRR and 95% CI: 1.1 (1.0, 1.2)
Glasson et al, 2004[34]	Western Australia, born between 1980 and 1995	Case-control	Western Australia 1980-95	PDD-NOS 84, controls 1313, siblings of cases 481	PE: ICD9	PDD-NOS	DSM	No association between PE and PDD-NOS: OR and 95% CI: 1.2 (0.5, 2.6)
Glasson et al, 2004[34]	Western Australia, born between 1980 and 1995	Case-control	Western Australia 1980-95	Asperger's 67, controls 1313, siblings of cases 481	PE: ICD9	Asperger's	DSM	No association between PE and Asperger's: OR and 95% CI: 1.3 (0.6, 3.0)
Eaton et al, 2001[62]	Offspring, age <15 years	Case-control	Denmark 1973-93	Asperger's Syndrome 279, reference population 102905	Eclampsia from Medical Birth Register	Asperger's Syndrome	ICD	Eclampsia v NT: No significant association RR: 1.06
Other HDP								
Study	**Population**	**Study design**	**Region, study period**	**Sample size**	**Diagnosis of HDP**	**Outcome measure**	**Assessment method**	**Main findings**
Böhm et al, 2017[22]	Millennium Cohort, age 7	Cohort	United Kingdom 2001-08	HDP 1069, No HDP 12431	HDP: self-reported (includes raised BP, eclampsia, PE or toxemia).	Behavioural difficulties	SDQ	No association between HDP and abnormal SDQ: OR and 95% CI: 0.94 (0.69, 1.29)

Polo-Kantola et al, 2014[35]	Singleton births in Finland between 1990-2005	Case-control	Finland 1990-2005	PDD 1602, Controls 6371	Maternal HT: (includes PE and pregnancy induced HT) ≥140/90mm Hg	PDD	ICD9 and ICD10	No association when results adjusted for SGA, other birth factors or neonatal treatment
Polo-Kantola et al, 2014[35]	Singleton births in Finland between 1990-2005	Case-control	Finland 1990-2005	Asperger's syndrome 1466 Controls 5839	Maternal HT: (includes PE and pregnancy induced HT) ≥140/90mm Hg	Asperger's syndrome	ICD9 and ICD10	No association OR and 95% CI: 1.03 (0.8, 1.4)
Robinson et al, 2009[67]	Offspring at age 2, 5, 8, 10 and 14 years	Cohort	Western Australia 1989-91	GH: 605 NT: 2119	GH: BP ≥140/90mm Hg after 24 weeks gestation	Behavioural problems in childhood and adolescence	CBCL	GH associated with increased risk of overall behavioural problems at age 8 and 14. OR and 95% CI: 1.40 (1.03, 1.91) 2.07 (1.35, 3.17) Also associated with increased risk of externalising behavioural problems at age 10 OR and 95% CI: 1.63 (1.13, 2.33)
Summary of HDP and intellectual disability studies								
Preeclampsia								
Study	**Population**	**Study design**	**Region, study period**	**Sample size**	**Diagnosis of HDP**	**Outcome measure**	**Assessment method**	**Main findings**
Griffith et al, 2011[63]	Live births in South Carolina between 1996-2002	Cohort	South Carolina 1996-2002	PE 5169, No PE 75697	PE or eclampsia: ICD9	Intellectual disability	Whether a child received special education or ID-related services from DDSN	PE associated with an increased risk of ID OR and 95% CI: 1.38 (1.16, 1.64)
Eaton et al, 2001[62]	Offspring, age <15 years	Case-control	Denmark 1973-93	Mental retardation 201, reference population 102905	Eclampsia from Medical Birth Register	Mental retardation	ICD	Eclampsia associated with statistically significant increased risk of mental retardation RR: 3.03

Other HDP								
Study	**Population**	**Study design**	**Region, study period**	**Sample size**	**Diagnosis of HDP**	**Outcome measure**	**Assessment method**	**Main findings**
Langridge et al, 2013[36]	All singleton births in Western Australia	Cohort	Western Australia 1984-99	Mild-moderate ID 4339, severe ID 237, unaffected children 376529	Pregnancy HT: PE and essential HT from MNS	Mild-moderate ID and severe ID	American Association on Mental Retardation classification system	Pregnancy HT associated with increased risk of mild-moderate ID: OR and 95% CI: 1.39 (1.25, 1.54) but not severe ID: 1.01 (0.64, 1.59)
Leonard et al, 2006[65]	Children born in Western Australia between 1983-92	Cohort	Western Australia 1983-92	HT 1379, No HT 238450	HT: ICD9	Intellectual disability	Mild-moderate ID: IQ 35 to 40 to 69 Severe ID: IQ<35 or 40 based on DSM-IV	No significant association. Mild-moderate ID: OR and 95% CI: 0.99 (0.58, 1.68) Severe ID: OR and 95% CI: 2.48 (0.79, 7.77)
Salonen, et al, 1984[150]	Children age 9-10 years living in one Finnish county (Kuopio)	Case-control	Eastern Finland 1979 and 1981	Mental retardation 136, Controls 122	HT during pregnancy: confirmed by a physician	Mental retardation	Screened using a standardised set of tests for mental performance	HT during pregnancy associated with increased risk of mental retardation RR and 95% CI: 6.1 (1.3, 28.9)

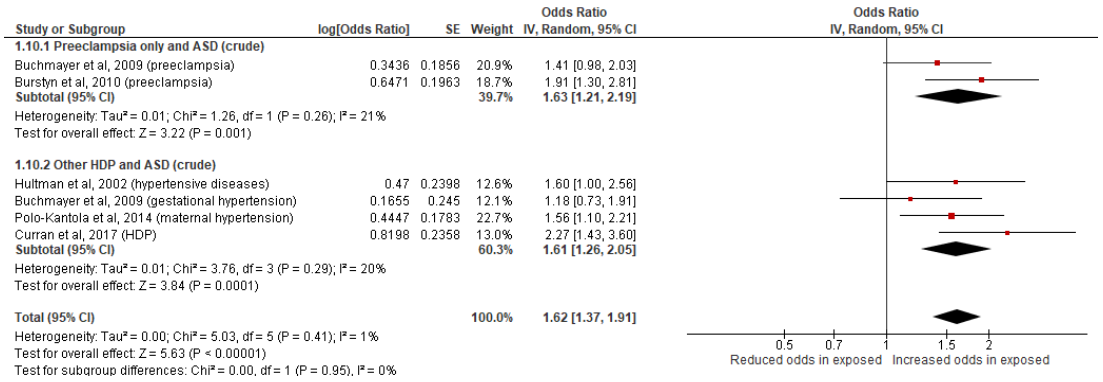
HDP=hypertensive disorder of pregnancy. PE=preeclampsia. BP=blood pressure. NT=normotensive. OR=odds ratio. 95% CI=95% confidence interval. IQ=intelligence quotient. WISC-R=Wechsler Intelligence Scale for Children-Revised. SBP=systolic blood pressure. DBP=diastolic blood pressure. CFQ=Cognitive Failures Questionnaire. DEX=Dysexecutive Questionnaire. MD=mean difference. ADHD=attention deficit/hyperactivity disorder. SNS=Support Needs System. HT=hypertension. PPVT-R=Peabody Picture Vocabulary Test-Revised. RCPM=Ravens Colored Progressive Matrices. BPP=Boerge Prien Prove. ICD=International Classification of Disease. RR=relative risk. WAIS=Wechsler Adult Intelligence Scale. PARCA-R=Parent Report of Children's Abilities-Revised. IUGR=Intrauterine growth restricted. MDI=Mental Developmental Index. PDI=Psychomotor Developmental Index. VLBW=very low birthweight. GH=gestational hypertension. PIH=pregnancy-induced hypertension. UTI=urinary tract infection. NR=not recorded. SGA=small for gestational age. SD=standard deviation. CBCL=Child Behaviour Checklists. IRR=incident rate ratio. PDD-NOS=Pervasive Developmental Disorder-Not Otherwise Specified. DSM=Diagnostic and Statistical Manual of Mental Disorders. GH=gestational hypertension. SDQ=Strengths and Difficulties Questionnaire. ID=intellectual disability. DDSN=Department of Disabilities and Special Needs. MNS=Midwives' Notification System.

Appendix 6: Funnel plot of published HDP-ASD studies that include adjusted estimates

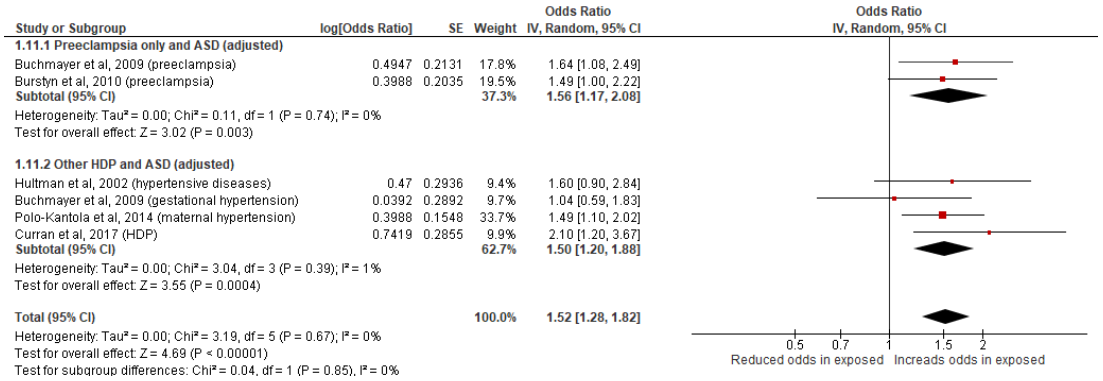


Appendix 7: Forest plots of the association between HDP and ASD, including only studies that adjust for maternal age and smoking and parity/birth order

Crude and partially adjusted estimates



Adjusted estimates



Appendix 8: Level of bias in ASD studies

Study	Selection bias	Exposure bias	Outcome bias	Confounding	Analytic bias	Attrition bias	Overall risk of bias
Curran et al, 2018[21]	Minimal: Sample selected from general population rather than a select group (Sample is representative of children born in the UK in 2000-01	Low: Recall < 1 year after birth	Minimal: Direct question to mother about outcome (doctor diagnosed maternal reporting)	Low: Certain confounders assessed - smoking during pregnancy, birth order, poverty, maternal ethnicity, age, education, depression, BMI, longstanding diabetes, longstanding HT	Low: Sample size calculation not performed, but all available eligible patients studied	Moderate: >20% attrition	Low
Walker et al, 2015[13]	Low: Sample from select group of population - only births in California who lived in catchment areas	Minimal: Direct questioning supplemented with medical records	Minimal: Previous ASD diagnoses were examined with validated measures	Low: Certain confounders assessed - maternal educational level, parity, pre-pregnancy obesity, age, sex, broad geographic regions within the study catchment areas	Low: Sample size calculation not performed, but all available eligible patients may have been studied	Minimal: Little to no attrition	Low
Polo-Kantola et al, 2014[35]	Minimal: Sample selected from general population rather than a select group	Low: Assessment of exposure from a dataset	Low: Assessment from administrative database	Low: Certain confounders assessed - maternal age, maternal smoking during pregnancy, number of previous births, maternal psychiatric history, sex, date of birth, place of birth	Minimal: Analyses appropriate for type of sample - conditional logistic regression	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Langridge et al, 2013[36]	Minimal: Consecutive unselected population	Low: Assessment of exposure from a dataset - Midwives' Notification System	Low: Assessment from administrative database	Low: Certain confounders assessed - birth year, maternal and pregnancy conditions (maternal diabetes, threatened abortion, asthma, UTI during pregnancy, placenta praevia, placenta abruption, other antepartum haemorrhage), socio-demographics (parity, maternal and paternal age group, maternal ethnicity, community-level socioeconomic status and community accessibility/remoteness), labour and delivery factors	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for (Registry data)	Low

				(preterm type, mode of delivery, breech, any complication of labour or delivery), neonatal outcomes (infant gender, resuscitation required at birth, percentage of optimal birthweight and head circumference)			
Mrozek-Budzyn et al, 2013[12]	Low: Sample from select group of population - cases from the one psychiatric outpatient clinic for children in the area. Controls identified through outpatient clinic records	Moderate: Medical records and/or interview with trained nurse 2-15 years after birth	Minimal: Cases identified using medical records from a psychiatric outpatient clinic for children	Moderate: Not assessed for confounders (but matched by year of birth, sex and general practitioners)	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Moderate
Nath et al, 2012[11]	Moderate: Sample selection ambiguous but sample may be representative	Moderate: Recall 1-5 years after birth	Minimal: DSM IV-TR	Moderate: Not assessed for confounders (but matched age)	Moderate: Sample size estimation unclear	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Moderate
Lyall et al, 2012[9]	Low: A select group of population - nurses only, high education status	Low: Indirect assessment - mailed questionnaire	Moderate: Assessment from "close-ended" questions	Low: Certain confounders assessed - race, marital status, income, spouse education, nurse's age at baseline, age at first birth, parity	Low: Sample size calculation not performed, but all available eligible patients studied	Moderate: >20% attrition	Moderate
Krakowiak et al, 2012[10]	Low: A select group of population - born in California, residing in specific catchment area	Minimal: Direct questioning supplemented with medical records	Minimal: Cases confirmed by trained clinician	Low: Certain confounders assessed - mother's age at delivery, race/ethnicity, education level, delivery payer, calendar time, age, gender, and regional centre catchment area	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: Little to no attrition	Low
Dodds et al, 2011[50]	Minimal: Consecutive unselected population - all live births	Low: Assessment of exposure from a dataset - Perinatal Database	Low: Assessment from administrative database	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all available	Minimal: All subjects from initiation of study to final outcome	Low

					eligible patients studied	assessment were accounted for	
Burstyn et al, 2010[31]	Minimal: Sample selected from general population rather than a select group	Low: Assessment of exposure from a dataset - delivery records held by Alberta Perinatal Health Programme	Low: Assessment from administrative database - ICD-9 codes linked to billing records	Low: Certain confounders assessed - maternal age, maternal weight, maternal height, pre-pregnancy diabetes, gestational diabetes, bleeding, smoking, poor weight gain, parity, mother's SES, presentation (breech etc.), type of labour, caesarean section, gestational age, birthweight, APGAR at 1 min and 5 mins, infant sex, birth year.	Low: Sample size calculation not performed, but all available eligible patients studied	Moderate: >20% attrition	Low
Mann et al, 2010[30]	Low: A select group of population - Medicaid Social Healthcare Programme	Low: Assessment of exposure from a dataset	Low: Assessment from administrative database	Low: Certain confounders assessed - maternal age, race, alcohol use, educational attainment, year of birth, child's sex, and diagnosis with a high risk condition (alcohol use, tobacco use, down syndrome, fragile X syndrome, brain anomaly) and birthweight	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Bilder et al, 2009[49]	Low: A select group of population - 8 year olds, vast majority white	Low: Birth certificate data	Low: Assessment from administrative database	Moderate: Not assessed for confounders for prenatal factors (but matched by gender and birth year)	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Buchmayer et al, 2009[14]	Low: A select group of population - overrepresentation of severe cases as inpatient care data available only	Low: Assessment of exposure from a dataset - Medical Birth Register	Low: Assessment from administrative database - Hospital Discharge Register	Low: Certain confounders assessed - parity, previous miscarriage, childless years, any maternal infection during pregnancy, season of delivery, diabetes mellitus, maternal age, smoking, maternal country of birth, whether the mother lived with the father, maternal schizophrenia, age,	Minimal: Analyses appropriate for type of sample - conditional logistic regression	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low

				gender, birth year, and birth hospital			
Larsson et al, 2005[148]	Minimal: Rational for case and control selection explained	Low: Assessment of exposure from a dataset - Danish Medical Birth Register	Low: Assessment from administrative database - Danish Psychiatric Register	Moderate: Not assessed for confounders (but matched by gender, birth year and age)	Minimal: Analyses appropriate for type of sample - conditional logistic regression	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Glasson et al, 2004[34]	Minimal: Sample selected from general population rather than a select group	Low: Assessment of exposure from a dataset - Maternal and Child Health Research Database	Low: Assessment from administrative database - Diagnosis and Service Delivery Records	Moderate: Not assessed for confounders (but matched by sex)	Minimal: Sample size calculation performed and adequate sample studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Hultman et al, 2002[15]	Low: A select group of population - overrepresentation of severe cases as inpatient care data available only	Low: Assessment of exposure from a dataset - Swedish Medical Birth Register	Low: Assessment from administrative database - Swedish Inpatient Register	Low: Certain confounders assessed - maternal age, parity, smoking during pregnancy, mother's country of birth, diabetes, pregnancy bleeding, mode of delivery, season of birth, gestational age, birthweight for gestational age, Apgar score at 5 minutes, congenital malformations, sex, year, and hospital of birth	Minimal: Analyses appropriate for type of sample - conditional logistic regression	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Eaton et al, 2001[62]	Low: A select group of population - cases were hospitalised (more severe)	Low: Assessment of exposure from a dataset - Medical Birth Register	Low: Assessment from administrative database - Danish Psychiatric Register	Low: Certain confounders assessed - gender and year of birth	Low: Sample size calculation not performed, but all available eligible patients studied (Does not provide 95% CI)	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Matsuishi et al, 1999[33]	Low: A select group of population - NICU survivors in a Japanese hospital	Minimal: Medical records	Minimal: Diagnosis confirmed by two paediatric neurologists who used DSM-III-R	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all available eligible patients studied	Low: <10% attrition	Low

Mason-Brothers et al, 1990[32]	Low: Epidemiological Survey of Utah	Minimal: Medical records	Minimal: Diagnosed by at least 2 clinicians using DSM-III	Moderate: Not assessed for confounders (but matched by sibling)	Moderate: Sample size estimation unclear	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Moderate
Deykin et al, 1980[147]	Low: A select group of population - Massachusetts, referred by 19 medical and educational facilities	Minimal: Medical records	High: Assessment from non-validated sources - parent-reported symptoms by age 6	Low: Certain confounders assessed - birth order, sibling	Moderate: Sample size estimation unclear	Low: Medical records located for 81% cases and 75% controls	Moderate

Appendix 9: Level of bias in ADHD studies

Study	Selection bias	Exposure bias	Outcome bias	Confounding	Analytic bias	Attrition bias	Overall risk of bias
Böhm et al, 2017[22]	Minimal: Sample selected from general population rather than a select group (when weighted, sample is representative of children born in the UK in 2000-01)	Low: Recall < 1 year after birth	Minimal: Direct question to mother about outcome (doctor diagnosed maternal reporting)	Low: Certain confounders assessed - alcohol during pregnancy, maternal education, maternal depression, maternal age, poverty status	Minimal: Analyses appropriate for type of sample - multivariate analysis	Moderate: >20% attrition	Low
Silva et al, 2014[39]	Minimal: Consecutive unselected population	Low: Assessment of exposure from a dataset - Midwives Notification System	Low: Assessment from administrative database - subjects dispensed stimulant medication from Monitoring of Drugs of Dependence System	Low: Certain confounders assessed - year of birth, gender, and socioeconomic status, marital status, parity, smoking, complications of pregnancy, onset of labor, augmentation of labor, complications of labor, type of delivery, child characteristics (gestational age, birthweight, average/small/large for gestational age) maternal age, Apgar at five minutes	Minimal: Analyses appropriate for type of sample - conditional logistic regression	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Cak and Gokler, 2013[48]	Low: Sample from select group of population (30-36 weeks gestation in one hospital)	Minimal: Medical records	Minimal: Schedule for Affective Disorders and Schizophrenia for School Aged Children-Present and Lifetime	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all available eligible patients studied	Moderate: >20% attrition but reasons for loss of follow up explained	Moderate

			Version semi-structured interview				
Getahun et al, 2013[40]	Minimal: Rational for case and control selection explained	Low: Assessment of exposure from a dataset - Perinatal Service System and inpatient/outpatient records	Minimal: Clinically diagnosed (1CD9) on at least 2 separate visits or 1 visit and 2 refills of ADHD medication	Low: Certain confounders assessed - age at diagnosis, maternal age, education, smoking during pregnancy, parity, prenatal care, household income, psychosocial disorder during pregnancy, child race/ethnicity, and gender	Minimal: Analyses appropriate for type of sample - conditional logistic regression	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Golmirzaei et al, 2013[42]	Low: A select group of population - sample of 4-11 year old school children, Southern Iran	High: Recall >5 years after birth	Minimal: Conner's Scales (those positive for ADHD were interviewed by psychiatrist using DSM-IV criteria)	Moderate: Not assessed for confounders (but matched by age)	Moderate: Sample size estimation unclear	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	High
Amiri et al, 2012[47]	Moderate: Sample selection ambiguous but sample may be representative	Moderate: Recall >5 years after birth but supplemented with medical documents where possible	Minimal: Direct question to parent about outcome using ADHD Rating Scale - Parent Version	Moderate: Not assessed for confounders (but matched by age)	Moderate: Sample size estimation unclear	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Moderate
Halmoy et al, 2012[45]	Minimal: Sample selected from general population rather than a select group	Low: Assessment of exposure from a dataset - Medical Birth Registry of Norway	Low: Assessment from administrative database - Adult patients who were approved for stimulant treatment in Norway during 1997-2005	Low: Certain confounders assessed - born in the same time period, year of birth, parity, age of mother at birth, educational level of mother and marital status of mother	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Ketzer et al, 2012[46]	Minimal: Rational for case and control selection explained	Moderate: Recall >5 years after birth (however	Minimal: Three stage process at outpatient clinic	Low: Certain confounders assessed - age,	Minimal: Analyses appropriate for type of sample -	Minimal: All subjects from initiation of study	Low

		supplemented with medical records in 38% of sample)		gender, agoraphobia (anxiety disorder), maternal ADHD and cigarettes/day during pregnancy	conditional logistic regression	to final outcome assessment were accounted for	
Gustafsson and Källén, 2011[44]	Minimal: Sample selected from general population rather than a select group	Low: Assessment of exposure from a dataset - Swedish Medical Birth Register	Low: Assessment from administrative database - Department of Child and Adolescent Psychiatry	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Mann and McDermott, 2011[41]	Low: A select group of population - Medicaid eligible women	Low: Assessment of exposure from a dataset - Medicaid billing data	Low: Assessment from administrative database - Medicaid billing data	Low: Certain confounders assessed - GU infection, infant race, maternal age and education, alcohol and tobacco use, infant sex, birthweight, and oldest age in Medicaid	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low

Appendix 10: Level of bias in other neurodevelopmental outcome studies

Study	Selection bias	Exposure bias	Outcome bias	Confounding	Analytic bias	Attrition bias	Overall risk of bias
Warshafsky et al, 2016[255]	Low: Sample from select group of population, based on residence	Minimal: Direct measurement of exposure from chart	Low: Indirect assessment (mailed questionnaire)	Low: Certain confounders assessed - MgSO4 usage, smoking, SES, sex, parity, breastfeeding, gestational age, IUGR	Moderate: Sample size estimation unclear	High: >20% attrition	Moderate
Johnson et al, 2015[69]	Low: Sample from four maternity centres, a midwife led unit and homebirths	Minimal: Exposure from maternity records	Low: Direct question to mother about outcome using Parent Report of Children's Abilities-Revised	Low: Certain confounders assessed - ethnic group, SES, sex, ethnic group, SES, sex, received breastmilk at discharge	Low: Sample size calculation not performed, but all available eligible patients studied	High: >20% attrition	Moderate
Morsing and Maršál, 2014[61]	Low: A select group of population - (preterm infants, Lund University Hospital)	Minimal: Exposure from clinical data	Minimal: Standardised test - Wechsler Scales IQ Test	Low: Matched for gestational age, gender and age at examination only	Moderate: Sample size estimation unclear	Minimal: Little to no attrition	Low
Heikura et al, 2013[277]	Low: All maternal healthcare centres in Oulu and Lapland, Northern Finland	Minimal: Structured questionnaire near time of exposure	Minimal: Psychometric tests collected from hospitals, institutions for children with intellectual disability, family counselling centres and school psychologists	Low: Certain confounders assessed - child's gender, family SES, maternal age, pre-pregnancy BMI, parity, birthweight	Low: Sample size calculation not performed, but all available eligible patients studied	Low: <10% attrition and reasons for loss of follow up not explained	Low
Tuovinen et al, 2013[278]	Low: Sample from select group of population, based on residence (Maternity Hospital, Helsinki, Finland)	Minimal: Exposure from hospital records	Low: Self-reported Cognitive Failures Questionnaire and Dysexecutive Questionnaire	Low: Certain confounders assessed - sex, length of gestation, weight, head circumference at birth, father's occupation in childhood, parity, mother's age, BMI at delivery, age at completion of questionnaire	Moderate: Sample size estimation unclear	Moderate: >20% attrition but reasons for loss of follow up explained	Moderate
Leitner et al, 2012[284]	Low: A select group - (IUGR)	Minimal: Direct questioning about	Minimal: Assessed by paediatric	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all	Low: All subjects from	Low

	born at one medical centre	exposure and medical records	neurologists and psychologists		available eligible patients studied	initiation of study to final outcome assessment were accounted for. (Not definite however as N only given for those with full data)	
Love et al, 2012[279]	Low: A select group of population - Aberdeen Grampian	Low: Assessment of exposure from a dataset (Aberdeen Maternity and Neonatal Databank)	Low: Assessment from administrative database (Support Needs System)	Low: Certain confounders assessed -maternal SES, induced labour, placental abruption, gestational age, birthweight	Minimal: Sample size calculation performed and adequate sample studied	Minimal: Little to no attrition	Low
Tuovinen et al, 2012[285]	Low: A select group of population - Maternity Hospital, Helsinki, Finland (Men only)	Minimal: Exposure from hospital records	Low: Administrative database - Finnish Defence Forces Basic Ability Test	Low: Certain confounders assessed - gestational age, weigh, head circumference at birth, year of birth, childhood SES, parity, mother's age and BMI at delivery, age and height at military service	Moderate: Sample size estimation unclear	Moderate: Only had data on 1196 out of 2786	Moderate
Tuovinen et al, 2012[286]	Low: A select group of population - Maternity Hospital, Helsinki, Finland (Men only)	Minimal: Exposure from hospital records	Low: Administrative database - Finnish Defence Forces Basic Ability Test	Low: Certain confounders assessed - length of gestation, weight and head circumference at birth, father's occupational status in childhood, parity, mother's age and BMI at delivery, age at testing, cognitive ability at 20 years, time interval between tests from 20-68 years, height at testing in late adulthood and blood pressure medication	Moderate: Sample size estimation unclear	Moderate: Only had data on 398 out of 931	Moderate
Whitehouse et al, 2012[280]	Low: A select group of population based on residence - Public antenatal	Minimal: Exposure from the chart and confirmed by	Minimal: Standardised tests - verbal ability and	Low: Certain confounders assessed - maternal age at conception, maternal education at pregnancy, household	Moderate: Sample size estimation unclear or only sub-sample	High: >20% attrition but reasons	Moderate

	clinic or surrounding private clinics in Perth, Western Australia. Sample may be over representative of lower SES group	obstetricians and midwives	non-verbal reasoning ability	income during pregnancy, maternal smoking and alcohol, maternal essential hypertension, maternal use of anti-hypertensive medication, spontaneous labour, parity, gestational age, birthweight, APGAR score, offspring sex, scores on McMaster Family Assessment device at 3 or 5 years of age	of eligible patients studied (Unclear what percentage of eligible participants included)		
Griffith et al, 2011[63]	Low: A select group of population based on residence and SES - South Carolina Medicaid data	Low: Assessment of exposure from a dataset - Medicaid billing files	Low: Assessment from administrative database - Dept. of Education and Dept. of Disabilities and Special Needs	Low: Certain confounders assessed - maternal age, white race, education, birth year, female sex, preterm status	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: Little to no attrition	Low
Leversen et al, 2011[149]	Low: All extremely preterm births in Norway	Low: Assessment of exposure from a dataset - Medical Birth Registry of Norway and registration forms	Minimal: Assessment by paediatrician at age 5	Low - Certain confounders assessed - gestational age, gender, illness severity score, small for gestational age, chorioamnionitis, prenatal steroids, multiple births, caesarean section, use of postnatal steroids, persistent ductus arteriosus, necrotizing enterocolitis, oxygen requirement at 36 weeks gestational age, retinopathy of prematurity, pathology on cerebral ultrasound, and high maternal education	Low: Sample size calculation not performed, but all available eligible patients studied	Low: 11-20% attrition	Low
Schlapbach et al, 2010[64]	Low: A select group of population - Zurich, preterm infants	Low: Assessment of exposure from a dataset - University Hospital Zurich neonatal database	Minimal: Performed routinely by paediatricians at age 2 years	Low: Certain confounders assessed - gestational age, birthweight, postnatal growth, mechanical ventilation, bronchopulmonary dysplasia	Low: Sample size calculation not performed, but all available eligible patients studied (small sample however: n=33 in each group)	Minimal: Little to no attrition	Low
Ehrenstein et al, 2009[281]	Low: A select group of population - Danish men who	Low: Assessment of exposure from a dataset - Danish	Minimal: Direct assessment using Boerge Prien Group	Low: Certain confounders assessed - small for gestational age, maternal age, parity,	Low: Sample size calculation not performed, but all	Minimal: Little to no attrition	Low

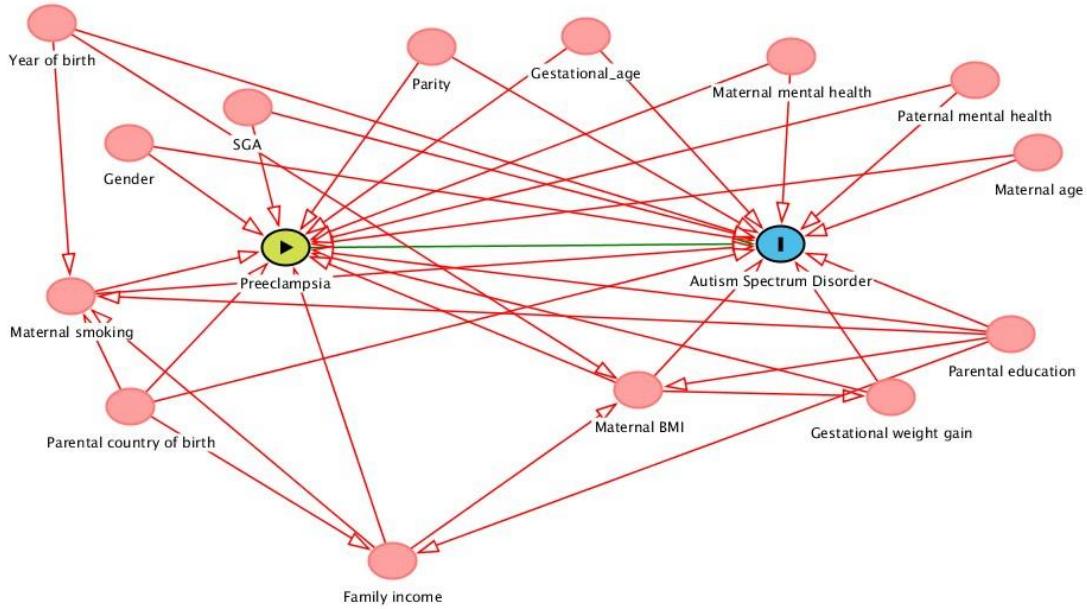
	presented for mandatory army fitness evaluation in the Fifth District	National Registry of Patients	Intelligence Test, converted to IQ scale	marital status, history of diabetes, conscript's year of birth, county of birth, birthweight, large for gestational age	available eligible patients studied		
Robinson et al, 2009[67]	Low: A select group of population -Public antenatal clinic or surrounding private clinics in Perth, Western Australia.	Minimal: Exposure from the chart by obstetricians and midwives in research team	Low: Parent Reported Child Behaviour Checklist	Low: Certain confounders assessed - gestational age, birthweight, maternal smoking in pregnancy, child sex, maternal experience of stressful events during pregnancy, maternal age at conception, maternal education in pregnancy, family income in pregnancy, presence of biological father during pregnancy, family functioning score	Low: Sample size calculation not performed, but all available eligible patients studied	High: >20% attrition - teenage and young mothers, those who did not live with child's father at birth, those who experienced high stress, those whose children had lower gestational age were less likely to remain in study	Low
Spinillo et al, 2009[66]	Low: A select group of population - preterm infants, single centre, Pavia, Italy	Minimal: Exposure from hospital records	Minimal: Bayley Scales of Infant Development by child neuropsychiatrist	Low: Certain confounders assessed - gestational age, proportion of expected birthweight, sex, umbilical artery, antenatal steroids	Low: Sample size calculation not performed, but all available eligible patients may have been studied	Minimal: <10% attrition	Low
Wu et al, 2009[293]	Minimal: Consecutive unselected population - all singleton born in Demar between 1978-2004	Low: Assessment of exposure from a dataset - Danish National Hospital Register	Low: Assessment of exposure from a dataset - Danish National Hospital Register	Low: Certain confounders assessed - infant sex, gestational age, parity, maternal age, maternal education, marriage status at birth, calendar year	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low

Silveira et al, 2007[68]	Low: A select group of population - very low birthweight infants in Hospital de Clinicas de Porto Alegre, Brazil	Minimal: Exposure from the chart	Minimal: Bayley Scales of Infant Development	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all available eligible patients studied	Moderate: >20% attrition but reasons for loss of follow up explained	Moderate
Leonard et al, 2006[65]	Minimal: Consecutive unselected population - Western Australia	Low: Assessment of exposure from a dataset - birth registry	Low: Assessment of exposure from a dataset - Disability Services Commission and education sources	Low: Aggregate SES measures	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: All subjects from initiation of study to final outcome assessment were accounted for	Low
Many et al, 2005[288]	Low: A select group of population - severe growth restriction	Minimal: Direct questioning (interview)	Minimal: Wechsler Preschool and Primary Scale of Intelligence	Moderate: Not assessed for confounders	Moderate: Sample size estimation unclear	Moderate: 11-20% attrition but reasons for loss of follow up not explained	Moderate
Lawlor et al, 2005[287]	Low: A select group of population - primary school attenders in Aberdeen, Scotland	Low: Assessment of exposure from a dataset - Maternal and Neonatal Database	Low: Assessment from administrative database - Aberdeen Childhood Development Survey linked to routine intelligence tests in primary schools	Moderate: Not assessed for confounders (not for HDP estimates, but associations between all complications of pregnancy and IQ attenuated towards the null when adjusted for parental characteristics)	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: Little to no attrition	Low
Cheng et al, 2004[57]	Low: A select group of population - very low birthweight, delivery before 32 weeks gestation	Minimal: Exposure from the chart	Minimal: Evaluated by psychiatrist - Bayley Scales of Infant Development	Low: Certain confounders assessed - parental education (unclear if there are others)	Low: Sample size calculation not performed, but all available eligible patients may have been studied	Minimal: Little to no attrition	Low

Many et al, 2003[60]	Low: A select group of population - Lis Maternity Hospital, Israel (children born growth restricted)	Minimal: Exposure from the chart	Minimal: Standardised IQ test	Low: Certain confounders assessed - gestational age, birthweight, neonatal complications	Low: Sample size calculation not performed, but all available eligible patients may have been studied	Low: 11-20% attrition	Low
McCowan et al, 2002[289]	Low: A select group of population - small for gestational age infants at Auckland Hospital, New Zealand	Minimal: Direct questioning (interview) by midwife	Minimal: Assessed by trained psychologist	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all available eligible patients may have been studied	Moderate: >20% attrition but reasons for loss of follow up explained	Moderate
Gray et al, 1998[290]	Low: A select group of population - very preterm infants, Mater Mother's Hospital, Brisbane, Australia	Minimal: Exposure from the chart	Minimal: Griffiths' Infant Ability Scale	Moderate: Not assessed for confounders	Minimal: Sample size calculation performed and adequate sample studied	Minimal: <10% attrition	Low
Spinillo et al, 1994[291]	Low: A select group of population - one clinical setting in Italy	Low: Assessment of exposure from a dataset	Low: Assessment from administrative database	Low: Certain confounders assessed - social class and maternal education	Low: Sample size calculation not performed, but all available eligible patients may have been studied	Minimal: <10% attrition	Low
Seidman et al, 1991[282]	Low: A select group of population - 17 year olds during assessment for drafting to Israel Defence Forces	Low: Assessment of exposure from a dataset - Jerusalem Perinatal Study	Low: Assessment from administrative database - Israel Defence Force records	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all available eligible patients may have been studied	Minimal: Little to no attrition	Low
Szymonowicz et al, 1987[58]	Low: A select group of population - very low birthweight infants at one centre	Low: Assessment of exposure from a dataset	Minimal: Bayley Scales	Moderate: Not assessed for confounders	Moderate: Sample size estimation unclear	Minimal: Little to no attrition	Moderate
Salonen, et al, 1984[150]	Low: A select group of population - All 9-10 year olds in one Finnish county in 1979-81	Minimal: Confirmed by physician during pregnancy	Low: Assessment from administrative database - records in local Developmental Defect Registries or screening in schools	Low: Certain confounders assessed- mother's age at birth, sibling with mental retardation or birth defect, parity, mode of birth, mother's smoking status during pregnancy	Low: Sample size calculation not performed, but all available eligible patients studied	Low: <10% attrition and reasons for loss of follow up not explained	Low

Winer et al, 1982[292]	Moderate: Sample selection process unclear	Minimal: Exposure from the chart	Minimal: Psychological testing (carried out blinded)	Moderate: Not assessed for confounders	Low: Sample size calculation not performed, but all available eligible patients may have been studied	Moderate: >20% attrition but reasons for loss of follow up explained	Moderate
Barker and Edwards, 1967[283]	Low: A select group of population - children in Birmingham who took the 'Eleven Plus' exam	Minimal: Exposure from the chart	Moderate: Eleven Plus exam (However, those in special schools or those in mainstream school but assessed as "borderline subnormal" excluded)	Moderate: Not assessed for confounders (but matched by sibpairs)	Low: Sample size calculation not performed, but all available eligible patients studied	Minimal: <10% attrition	Moderate

Appendix 11: Directed acyclic graph used to identify potential confounders in Swedish National Registers for <u>preeclampsia-ASD study</u>



Appendix 12: Description of confounders in Swedish National Registers

Year of birth: 1982-2010 for ASD study, 1990-2010 for ADHD study.

Infant sex: Male and female.

Maternal age: Categorised as <20years, 20-29 years, 30-39, and ≥40 years.

Parental country of birth: Categorised as "Sweden", "other Nordic country," and "other country".

Parity: Number of pregnancies reaching viable gestational age.

Maternal smoking status: Categorised as "no smoking," "smoking 1-9 cigarettes a day," and "smoking ≥10 cigarettes a day".

Body mass index (BMI) at first antenatal visit: Categorised as <20, 20-24.9, 25-29.9 and ≥30.

Optimal gestational weight gain: This was established for each maternal BMI category based on significant risk estimates of adverse maternal and fetal outcomes[230].

Maternal and paternal depression: ICD-8: 29600, 3004; ICD-9: 296B, 311, 300E; ICD-10: F32, F33.

Maternal and paternal bipolar disorder: ICD-8: 29610-29630; ICD-9: 296A,C-E; ICD-10: F30-31.

Maternal and paternal non-affective psychiatric disorders: ICD-8: 295, 297, 298 [excluding 29800 and 29810], 29999; ICD-9: 295, 297, 298 [excluding 298A and 298B]; ICD-10: F20-29.

Family income: Disposable income in the household the year the child was born. This was divided into quintiles, ranging from "low income" to "high income".

Parental level of education: Available since 1990, parental level of education was categorised as "pre-high school," "high school," and "post high school".

Appendix 13: Full description of results of sensitivity analyses examining association between preeclampsia and ASD

When the study population was restricted to 1987-2010, preeclampsia was associated with a 25% increase in the likelihood of ASD, compared to those unexposed to preeclampsia (HR: 1.25, 95% CI: 1.19, 1.31). Similarly, excluding births after 2006 did not materially change results (HR: 1.24, 95% CI: 1.18, 1.30).

Fully adjusted results of the sensitivity analysis suggested that preeclampsia in those born at ≥34 weeks' gestational age was associated with an 18% increase in the likelihood of ASD (HR: 1.18, 95% CI: 1.13, 1.24) when compared to those unexposed

to preeclampsia, and born at a similar gestational age. The fully adjusted result for preeclampsia in those born at <34 weeks' gestational age (used as a proxy for preeclampsia with severe features) was 2.04 (95% CI: 1.81, 2.30) when compared to non-exposure to preeclampsia in those born at ≥34 weeks' gestation. The HR for a preeclampsia-ASD relationship, excluding those with chronic hypertension, was 1.26 (95% CI: 1.20, 1.31); and including those with both preeclampsia and chronic hypertension: 0.91 (95% CI: 0.63, 1.31). The fully adjusted HR for preeclampsia (excluding those with family history of mental illness) was 1.28 (95% CI: 1.22, 1.35). Including caesarean section in the multivariate model resulted in a HR of 1.21 (95% CI: 1.15, 1.26). Preeclampsia with a low/intermediate APGAR score at five minutes increased the likelihood of ASD by 30% compared to non-exposure to preeclampsia and low/intermediate score. Finally, preeclampsia among mothers <20 years of age and mothers with a BMI of <20 was associated with the highest odds of ASD (HR: 1.37 and 1.29 respectively) compared to those of similar maternal age and BMI at first antenatal visit. Adjusted subgroup analysis suggested a statistically significant increase in the likelihood of ASD at all gestational ages when compared to non-exposure to preeclampsia in those born at ≥37 weeks' gestation. When adjusted for potential confounders, exposure to preeclampsia was associated with a 25% increase in the odds of ASD in both male and female offspring (HR: 1.25, 95% CI: 1.18, 1.32) and (HR: 1.25, 95% CI: 1.16, 1.35 respectively). (see **Appendix 14** and **Appendix 15** below).

Appendix 14: Sensitivity analyses examining the association between preeclampsia and ASD among singleton live births in Sweden between 1982 and 2010

Variable	Exposed cases	Model 1 HR (95% CI)[a]	Model 2 HR (95% CI)[b]
Preeclampsia (Study population restricted to 1987-2010)	1843	1.37 (1.31, 1.44)	1.25 (1.19, 1.31)
Preeclampsia (excluding births after 2006)	1830	1.35 (1.29, 1.41)	1.24 (1.18, 1.30)
Preeclampsia (born ≥34 weeks' gestational age)[e]	1755	1.30 (1.24, 1.36)	1.18 (1.13, 1.24)
Preeclampsia (born <34 weeks' gestational age)[e]	269	2.23 (1.97, 2.51)	2.04 (1.81, 2.30)
Preeclampsia without chronic hypertension[f]	1996	1.37 (1.31, 1.43)	1.26 (1.20, 1.31)
Preeclampsia with chronic hypertension[f]	28	1.03 (0.71, 1.49)	0.91 (0.63, 1.31)
Preeclampsia excluding those with family history of mental illness[g]	1575	1.40 (1.33, 1.48)	1.28 (1.22, 1.35)[c]
Preeclampsia-ASD (+adjusted for caesarean section)	2024	1.36 (1.31, 1.43)	1.21 (1.15, 1.26)[d]
Preeclampsia with low/intermediate APGAR at 5 minutes[h]	87	1.35 (1.09, 1.69)	1.30 (1.04, 1.62)
Preeclampsia by maternal age[i]			
<20	99	1.46 (1.19, 1.79)	1.37 (1.12, 1.68)
20-29	1053	1.32 (1.24, 1.40)	1.22 (1.15, 1.30)
30-39	798	1.41 (1.31, 1.51)	1.29 (1.20, 1.38)
≥40	74	1.19 (0.94, 1.50)	1.11 (0.88, 1.40)
Preeclampsia by BMI at first antenatal visit[j]			
<20	117	1.31 (1.09, 1.57)	1.29 (1.07, 1.55)
20-24.9	606	1.33 (1.22, 1.44)	1.27 (1.17, 1.38)
25-29.9	392	1.16 (1.04, 1.28)	1.11 (1.01, 1.23)
≥30	372	1.22 (1.09, 1.35)	1.18 (1.06, 1.31)

Abbreviations: HR=hazard ratio. 95% CI=95% confidence interval. BMI=body mass index.
[a]Adjusted for year of birth.
[b]Adjusted for year of birth, infant sex, maternal age, maternal and paternal country of birth, birth order, parental depression, bipolar disorder and non-affective psychiatric disorders, maternal smoking status, BMI at first antenatal visit, gestational weight gain, family income and parental level of education.
[c]Adjusted for same potential confounders as above with the exception of parental mental health.
[d]Adjusted for same potential confounders as 'b' above, in addition to adjusting for caesarean section.
[e]Reference=deliveries ≥34 weeks' gestational age in mothers with no preeclampsia. [f]Reference=no preeclampsia/no chronic hypertension. [g]Reference=no preeclampsia/no family history of mental illness. [h]Reference=no preeclampsia/low/intermediate APGAR. [i]Reference=no preeclampsia at corresponding maternal age. [j]Reference=no preeclampsia with corresponding BMI.

Appendix 15: Association between preeclampsia and ASD among singleton live births in Sweden between 1982 and 2010 by gestational age and infant sex

Variable	Total Population	Exposed Cases	Model 1 HR (95% CI)[a]	Model 2 HR (95% CI)[b]
Gestational age[c]	**N (%)**		**Preeclampsia**	**Preeclampsia**
<34 weeks	32,332 (1.1)	269	2.25 (2.00, 2.54)	2.05 (1.82, 2.31)
34 weeks	17,162 (0.6)	57	1.32 (1.02, 1.71)	1.19 (0.92, 1.54)
35 weeks	29,982 (1.1)	83	1.44 (1.16, 1.78)	1.32 (1.06, 1.64)
36 weeks	60,016 (2.1)	153	1.57 (1.34, 1.84)	1.42 (1.21, 1.67)
37 weeks	141036 (5.0)	226	1.41 (1.24, 1.61)	1.29 (1.13, 1.47)
38 weeks	386963 (13.6)	319	1.37 (1.23, 1.53)	1.26 (1.13, 1.41)
39 weeks	657765 (23.2)	351	1.28 (1.15, 1.42)	1.17 (1.06, 1.30)
40 weeks	799752 (28.2)	316	1.21 (1.08, 1.35)	1.12 (1.00, 1.25)
>40 weeks	712440 (25.1)	240	1.18 (1.04, 1.34)	1.03 (0.91, 1.17)
Infant sex				
Male[d]	1460940 (51.4)	1386	1.36 (1.29, 1.43)	1.25 (1.18, 1.32)
Female[e]	1381290 (48.6)	638	1.36 (1.26, 1.47)	1.25 (1.16, 1.35)

Abbreviations: HR=hazard ratio. 95% CI=95% confidence interval.
[a]Adjusted for year of birth.
[b]Adjusted for year of birth, infant sex, maternal age, maternal and paternal country of birth, birth order, parental depression, bipolar disorder and non-affective psychiatric disorders, maternal smoking status, BMI at first antenatal visit, gestational weight gain, family income and parental level of education.
[c]Reference=no preeclampsia/born at ≥37 weeks' gestation.
[d]Reference=no preeclampsia in males.
[e]Reference=no preeclampsia in females.

Appendix 16: E-value worked example for preeclampsia-ADHD[235]

E-Value for effect estimate:	= RR + sqrt[RR x (RR-1)]
	= 1.13 + sqrt[1.13 x (1.13-1)]
	= 1.13 + 0.38
	= 1.51
E-Value for lower limit (LL) of CI:	= LL + sqrt[LL x (LL-1)]
	= 1.05 + sqrt[1.05 x (1.05-1)]
	= 1.05 + 0.23
	= 1.28

Appendix 17: Full description of results of sensitivity analyses examining association between preeclampsia and ADHD

When we excluded women who had preeclampsia in their first pregnancy, the adjusted HR was 1.21 (95% CI: 1.13, 1.30). When we restricted the study population to 2001-2010 and 1994-2010 the HR was 1.21 (95% CI: 1.16, 1.28) and 1.14 (95% CI: 1.10, 1.18) respectively. The adjusted HR for preeclampsia (excluding chronic hypertension) and preeclampsia (with chronic hypertension) were 1.15 (95% CI: 1.12, 1.19) and 1.18 (95% CI: 0.93, 1.50) respectively. The HR for preeclampsia (excluding those with a family history of mental illness was 1.16 (95% CI: 1.12, 1.21). The HR for preeclampsia (with low/intermediate APGAR at 5 minutes) was 1.13 (95% CI: 0.94, 1.36) when compared to non-exposure to preeclampsia in those with a low/intermediate APGAR score. Results of the subgroup analysis by maternal age were not significantly different from each other. Similarly, results of the subgroup analysis by BMI at first antenatal visit did not significantly differ from each other. The HR for preeclampsia-ADHD in males was 1.18 (95% CI: 1.14, 1.23) compared to non-exposure to preeclampsia in males. The HR for preeclampsia-ADHD in females was 1.10 (1.04, 1.17) compared to non-exposure to preeclampsia in females. Finally, exposure to preeclampsia in males was associated with a 9% increase in likelihood of ADHD when compared to exposure to preeclampsia in females. (see **Appendix 18** below).

Appendix 18: Sensitivity analyses examining the association between preeclampsia and ADHD among singleton live births in Sweden between 1990 and 2010

Variable	Exposed cases	Model 1 HR (95% CI)[a]	Model 2 HR (95% CI)[b]
Preeclampsia (excluding women who had preeclampsia in first pregnancy)	726	1.29 (1.20, 1.39)	1.21 (1.13, 1.30)
Preeclampsia (Study population restricted to 2001-2010)	1660	1.35 (1.29, 1.42)	1.21 (1.16, 1.28)
Preeclampsia (Study population restricted to 1994-2010)	3237	1.22 (1.18, 1.27)	1.14 (1.10, 1.18)
Preeclampsia excluding chronic hypertension[d]	3872	1.21 (1.18, 1.25)	1.15 (1.12, 1.19)
Preeclampsia with chronic hypertension[d]	69	1.31 (1.04, 1.66)	1.18 (0.93, 1.50)
Preeclampsia excluding those with family history of mental illness[e]	2803	1.23 (1.19, 1.28)	1.16 (1.12, 1.21)[c]
Preeclampsia with low/intermediate APGAR at 5 minutes[f]	123	1.17 (0.97, 1.41)	1.13 (0.94, 1.36)
Preeclampsia by maternal age[g]			
<20	235	1.19 (1.04, 1.36)	1.19 (1.05, 1.36)
20-29	2145	1.15 (1.11, 1.20)	1.12 (1.07, 1.17)
30-39	1439	1.26 (1.20, 1.33)	1.20 (1.14, 1.26)
≥40	122	1.25 (1.04, 1.49)	1.18 (0.98, 1.42)
Preeclampsia by BMI at first antenatal visit[h]			
<20	192	1.19 (1.03, 1.37)	1.24 (1.08, 1.44)
20-24.9	1064	1.09 (1.03, 1.16)	1.09 (1.03, 1.16)
25-29.9	874	1.12 (1.04, 1.19)	1.13 (1.06, 1.21)
≥30	877	1.10 (1.02, 1.17)	1.13 (1.06, 1.21)
Infant sex			
Males[i]	2655	1.25 (1.21, 1.30)	1.18 (1.14, 1.23)
Females[j]	1286	1.14 (1.07, 1.20)	1.10 (1.04, 1.17)
Males v Females[k]	2655	1.10 (1.03, 1.18)	1.09 (1.02, 1.17)

Abbreviations: HR=hazard ratio. 95% CI=95% confidence interval. SGA=small for gestational age. BMI=body mass index.
[a]Adjusted for year of birth.
[b]Adjusted for year of birth, infant sex, maternal age, maternal and paternal country of birth, firstborn, parental depression, bipolar disorder, and non-affective psychiatric disorders, family income, maternal smoking status, BMI at first antenatal visit, gestational weight gain and parental level of education.
[c]Adjusted for same potential confounders as 'b' above with the exception of parental depression, bipolar disorder, and non-affective psychiatric disorders
[d]Reference=no preeclampsia/no chronic hypertension [e]Reference=no preeclampsia/no family history of mental illness. [f]Reference=no preeclampsia/with low/intermediate APGAR. [g]Reference=no preeclampsia at corresponding maternal age. [h]Reference=no preeclampsia with corresponding BMI. [i]Reference=non-exposure to preeclampsia in male offspring. [j]Reference= non-exposure to preeclampsia in female offspring. [k]Reference=exposure to preeclampsia in females.
Missing data on BMI at first antenatal visit: N= 480339 (including 934 cases of ADHD)

Appendix 19: Summary of data collection process in GUI study

Wave (age at time of wave)	Data collected	Method of collection
Wave 1 (age 9-months)	1. Preeclampsia (and Preeclampsia+SGA)	Face-to-face interview with Primary Caregiver
	2. Potential confounding factors	Face-to-face interview with Primary Caregiver
	3. ASQ	Face-to-face interview with Primary Caregiver
Wave 2 (age 3 years)	SDQ	Face-to-face interview with Primary Caregiver
Wave 3 (age 5 years)	SDQ	Face-to-face interview with Primary Caregiver
	SDQ	Postal survey completed by study child's teacher
Wave 4 (age 7-8 years)	SDQ	Postal survey completed by Primary Caregiver
Abbreviations: ASQ=Ages and Stages Questionnaire. SDQ=Strengths and Difficulties Questionnaire.		

Appendix 20: Repeated measures analysis examining the association between preeclampsia and emotional/behavioural problems (by SDQ domain) among singleton live births in Ireland

Adjusted model[a] **Emotional**	**Mean trajectory (95% CI) (No Preeclampsia)**	**Mean trajectory (95% CI) (Preeclampsia)**	**Mean difference in trajectory (95% CI) comparing no preeclampsia to preeclampsia**
Age 3 SDQ	1.71 (1.30, 2.12)	1.79 (1.36, 2.21)	-0.08 (-0.19, 0.03)
Change SDQ Age 5	-0.61 (-2.23, 1.02)	-0.44 (-2.07, 1.20)	-0.17 (-0.03, -0.31)
Change SDQ Age 7-8	2.93 (-0.65, 6.51)	2.91 (-0.68, 6.49)	0.03 (0.19, 0.24)
Age 7-8 SDQ	4.03 (0.36, 7.70)	4.26 (0.58, 7.94)	-0.22 (-0.01, -0.44)
Conduct			
Age 3 SDQ	2.70 (2.29, 3.11)	2.78 (2.36, 3.21)	-0.08 (-0.21, 0.04)
Change SDQ Age 5	-0.69 (-0.73, -0.66)	-0.61 (-0.75, -0.47)	-0.08 (-0.23, 0.06)
Change SDQ Age 7-8	-0.09 (-0.13, -0.04)	-0.31 (-0.48, -0.14)	0.22 (0.04, 0.40)
Age 7-8 SDQ	1.92 (1.51, 2.33)	1.87 (1.43, 2.31)	0.05 (-0.12, 0.22)
Hyperactivity			
Age 3 SDQ	5.73 (5.11, 6.36)	5.66 (5.01, 6.30)	0.08 (-0.09, 0.25)
Change SDQ Age 5	0.57 (-1.68, 2.81)	0.88 (-1.37, 3.14)	-0.32 (-0.51, -0.12)
Change SDQ Age 7-8	0.67 (-3.28, 4.62)	0.48 (-3.48, 4.44)	-0.19 (-0.05, 0.43)
Age 7-8 SDQ	6.97 (2.91, 11.03)	7.02 (2.95, 11.09)	-0.05 (-0.30, 0.19)
Peer Problems			
Age 3 SDQ	2.36 (1.98, 2.75)	2.39 (1.99, 2.79)	-0.03 (-0.14, 0.08)
Change SDQ Age 5	0.19 (-1.24, 1.63)	0.29 (-1.15, 1.73)	-0.10 (-0.23, 0.03)
Change SDQ Age 7-8	-0.33 (-3.15, 2.48)	-0.51 (-3.33, 2.31)	0.17 (0.01, 0.35)
Age 7-8 SDQ	2.22 (-0.58, 5.02)	2.17 (-0.64, 4.98)	0.05 (-0.11, 0.21)
Prosocial Behaviour[b]			
Age 3 SDQ	7.39 (6.90, 7.89)	7.49 (6.98, 7.80)	-0.09 (-0.23, 0.04)
Change SDQ Age 5	0.75 (-0.97, 2.47)	0.66 (-1.07, 2.39)	0.09 (-0.06, 0.24)
Change SDQ Age 7-8	1.73 (-1.46, 4.91)	1.80 (-1.39, 4.99)	-0.07 (-0.27, 0.13)
Age 7-8 SDQ	9.87 (6.69, 13.04)	9.94 (6.76, 13.12)	-0.07 (-0.26, 0.11)

Abbreviations: 95% CI=95% confidence interval. SDQ=Strengths and Difficulties Questionnaire.

[a]Adjusted for maternal education, maternal age, maternal ethnicity, maternal body mass index (BMI) at time of interview, family social class, gestational diabetes, and infant sex.

[b]Prosocial Behaviour is reversed scored (i.e. higher scores indicate more positive outcomes).

Appendix 21: Assessment of repeated measures model fit from GUI study. Comparison of predicted values (from multilevel model) with observed values at age 3, 5 and 7-8 years.

	Mean observed (SD)	**Mean predicted (SD)**	**Mean difference (observed - predicted)**	**95% level of agreement between observed and predicted**
SDQ score				
Age 3	7.77 (4.53)	7.78 (2.95)	-0.01	-4.31, 4.31
Age 5	7.18 (4.75)	7.23 (3.34)	-0.05	-4.05, 4.05
Age 7-8	7.10 (5.30)	7.44 (3.72)	-0.33	-3.63, 3.63
Abbreviations: SD=standard deviation. 95% CI=95% confidence interval. SDQ=Strengths and Difficulties Questionnaire.				

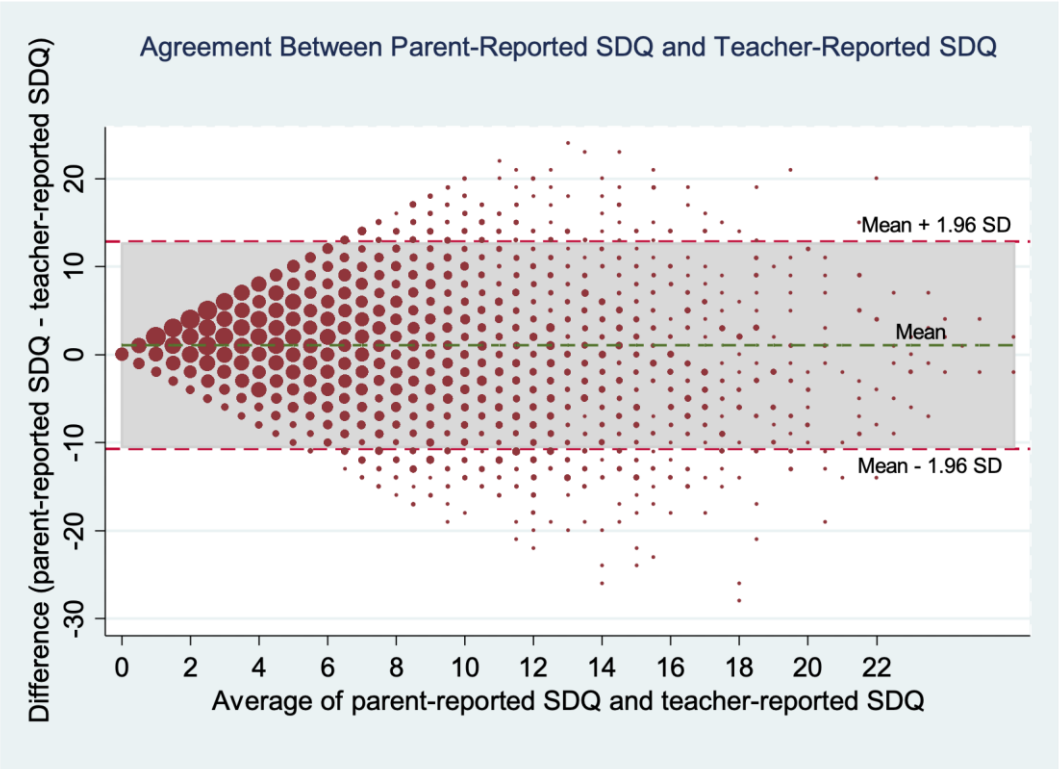
Appendix 22: Sensitivity analysis examining associations between preeclampsia and child development and emotional/behavioural problems among singleton live births in Ireland

Failure of ASQ domains **(including primiparous women only)**	**Adjusted model[a] OR (95% CI)**
Communication	1.32 (0.80, 2.19)
Gross Motor	1.15 (0.78, 1.71)
Fine Motor	0.73 (0.45, 1.17)
Problem Solving	0.74 (0.48, 1.14)
Personal Social	1.25 (0.84, 1.86)
Abnormal SDQ age 3 years **(including primiparous women only)**	
Emotional	1.38 (0.74, 2.57)
Conduct	1.06 (0.76, 1.48)
Hyperactivity	0.93 (0.56, 1.55)
Peer Problems	0.69 (0.42, 1.14)
Prosocial Behaviour	1.32 (0.60, 2.91)
Abnormal SDQ age 5 years **(including primiparous women only)**	
Emotional	1.75 (1.09, 2.82)
Conduct	0.78 (0.47, 1.30)
Hyperactivity	1.64 (1.11, 2.42)
Peer Problems	1.53 (0.90, 2.60)
Prosocial Behaviour	1.21 (0.42, 3.55)
Abnormal SDQ age 7/8 years **(including primiparous women only)**	
Emotional	1.18 (0.70, 1.99)
Conduct	0.99 (0.53, 1.89)
Hyperactivity	0.98 (0.52, 1.84)
Peer Problems	1.02 (0.52, 1.97)
Prosocial Behaviour	0.30 (0.07, 1.38)
Total SDQ score age 5 years (Maternal reported)	**Adjusted model[a] Coefficient (95% CI)**
Preeclampsia	0.79 (0.26, 1.31)
Total SDQ score age 5 years (Teacher reported)	
Preeclampsia	0.71 (0.12, 1.31)

Abbreviations: OR=odds ratio. 95% CI=95% confidence interval. ASQ=Ages and Stages Questionnaire. SDQ=Strengths and Difficulties Questionnaire.

[a]Adjusted for maternal education, maternal age, maternal ethnicity, maternal body mass index (BMI) at time of interview, family social class, gestational diabetes and infant sex.

Appendix 23: Bland-Altman agreement plot comparing parent-reported SDQ scores to teacher-reported SDQ scores at age 5 years in GUI Study



Appendix 24: Sensitivity analysis of repeated measures analysis examining the association between preeclampsia/SGA and emotional/behavioural problems (using total SDQ Score) among singleton live births in Ireland

(Restricting study population to Irish/other white background)

Model 1[a]	**Mean trajectory (95% CI) (No Preeclampsia/No SGA)**	**Mean trajectory (95% CI) (Preeclampsia+SGA)**	**Mean difference in trajectory (95% CI) comparing no preeclampsia/no SGA to preeclampsia+SGA**
Age 3 SDQ	11.56 (10.05, 13.06)	11.89 (10.09, 13.68)	-0.33 (-1.31, 0.64)
Change SDQ Age 5	-4.16 (-10.25, 1.93)	-3.42 (-9.59, 2.75)	-0.74 (-1.78, 0.30)
Change SDQ Age 7-8	1.42 (-0.21, 3.05)	0.51 (-1.66, 2.67)	0.91 (-0.57, 2.40)
Age 7-8 SDQ	8.81 (2.39, 15.24)	8.98 (2.37, 15.58)	-0.16 (-1.73, 1.40)
Abbreviations: 95% CI=95% confidence interval. SDQ=Strengths and Difficulties Questionnaire. SGA=small for gestational age. [a]Adjusted for maternal education, maternal age, maternal ethnicity, maternal body mass index (BMI) at time of interview, family social class, gestational diabetes, and infant sex.			

Appendix 25: Sensitivity analysis examining associations between preeclampsia and child development and emotional/behavioural problems among singleton live births in Ireland by gestational age

Failure of ASQ domains (Preeclampsia, born <37 weeks' gestation)[b]	**Adjusted model**[a] **OR (95% CI)**
Communication	3.10 (1.49, 6.45)
Gross Motor	2.80 (1.63, 4.85)
Fine Motor	1.54 (0.77, 3.07)
Problem Solving	1.74 (0.96, 3.16)
Personal Social	1.42 (0.77, 2.61)
Failure of ASQ domains (Preeclampsia, born ≥37 weeks' gestation)[b]	
Communication	0.96 (0.64, 1.46)
Gross Motor	0.93 (0.70, 1.23)
Fine Motor	0.88 (0.63, 1.22)
Problem Solving	0.80 (0.60, 1.08)
Personal Social	0.92 (0.70, 1.20)
Abnormal SDQ age 3 years (Preeclampsia, born <37 weeks' gestation)[b]	
Emotional	1.43 (0.46, 4.49)
Conduct	0.75 (0.40, 1.38)
Hyperactivity	1.40 (0.64, 3.08)
Peer Problems	1.07 (0.46, 2.49)
Prosocial Behaviour	1.82 (0.60, 5.53)
Abnormal SDQ age 3 years (Preeclampsia, born ≥37 weeks' gestation)[b]	
Emotional	1.28 (0.77, 2.13)
Conduct	1.10 (0.87, 1.40)
Hyperactivity	0.87 (0.59, 1.29)
Peer Problems	0.85 (0.57, 1.25)
Prosocial Behaviour	1.06 (0.59, 1.90)
Abnormal SDQ age 5 years (Preeclampsia, born <37 weeks' gestation)[b]	
Emotional	2.39 (1.07, 5.37)
Conduct	1.45 (0.69, 3.04)
Hyperactivity	2.22 (1.12, 4.39)
Peer Problems	2.56 (1.15, 5.69)
Prosocial Behaviour	1.60 (0.33, 7.81)
Abnormal SDQ age 5 years (Preeclampsia, born ≥37 weeks' gestation)[b]	
Emotional	1.46 (0.96, 2.20)
Conduct	1.08 (0.76, 1.54)
Hyperactivity	1.50 (1.11, 2.03)
Peer Problems	1.31 (0.86, 1.99)
Prosocial Behaviour	1.42 (0.72, 2.80)
Abnormal SDQ age 7/8 years (Preeclampsia, born <37 weeks' gestation)[b]	
Emotional	1.52 (0.59, 3.88)
Conduct	0.58 (0.19, 1.79)
Hyperactivity	0.49 (0.11, 2.10)
Peer Problems	1.14 (0.35, 3.74)
Prosocial Behaviour	-
Abnormal SDQ age 7/8 years (Preeclampsia, born ≥37 weeks' gestation)[b]	
Emotional	1.11 (0.73, 1.69)
Conduct	0.90 (0.54, 1.50)
Hyperactivity	0.87 (0.52, 1.45)
Peer Problems	0.69 (0.39, 1.21)
Prosocial Behaviour	0.67 (0.24, 1.89)
Abbreviations: OR=odds ratio. 95% CI=95% confidence interval. ASQ=Ages and Stages Questionnaire. SDQ=Strengths and Difficulties Questionnaire.	

[a]Adjusted for maternal education, maternal age, maternal ethnicity, maternal body mass index (BMI) at time of interview, family social class, gestational diabetes, and infant sex.
[b]Reference=no preeclampsia/born ≥37 weeks' gestation).
Reason for empty cells: n too small to estimate.

Appendix 26: PhD-related papers

Available from the following DOI:

1. 10.1136/bmjopen-2017-018313

2. 10.1001/jamapsychiatry.2018.0854

3. 10.1016/j.ijdevneu.2018.10.004

4. 10.1111/jcpp.13127

5. 10.1111/acps.13162

6. 10.1111/acps.13180

7. 10.1007/s10995-020-02921-7

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